MENINGOENCEPHALOMYELITIS OF UNKNOWN AETIOLOGY IN DOGS

A diagnostic, therapeutic and prognostic challenge

Ine Cornelis

Dissertation submitted in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD) in Veterinary Sciences, Small Animal Department, Faculty of Veterinary Medicine

Ghent University

2017

Promotoren: Dr. Sofie Bhatti

Prof. Dr. Luc Van Ham

Dr. Ingrid Gielen

Dr. Steven De Decker

This thesis was performed in collaboration with:

The Royal Veterinary College, University of London.

Ine Cornelis

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Universiteit Gent, Faculteit Diergeneeskunde
Vakgroep Kleine Huisdieren

Coverfoto's: Sali en Wiebe door Fotostudio Wo

Printing of this thesis was enabled by the generous support of:

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List of abbreviations

^{1}H MRS	Single Voxel Proton Magnetic Resonance Spectroscopy
ANNPE	Acute Noncompressive Nucleus Pulposus Extrusion
AUC	Area Under the Curve
BAR	Bright Alert Responsive
CBC	Complete Blood Count
CDV	Canine Distemper Virus
CI	Confidence Interval
CNS	Central Nervous System
CRI	Constant Rate Infusion
CSF	Cerebrospinal Fluid
EME	Eosinophilic Meningoencephalitis
FCEM	Fibrocartilagenous Embolic Myelopathy
FDG-PET	Fluorodeoxyglucose - Positron Emission Tomography
FDR	False Discovery Rate
FLAIR	Fluid Attenuation Inversion Recovery
GME	Granulomatous Meningoencephalomyelitis
ICP	Intracranial Pressure
IM	Ischaemic Myelopathy
IQR	Interquartile Ranges
IV	Intravenous
MRI	Magnetic Resonance Imaging
MST	Median Survival Time
MUA	Meningoencephalomyelitis of Unknown Aetiology
MUO	Meningoencephalomyelitis of Unknown Origin
NE	Necrotizing Encephalitis

NIME	Non-Infectious Meningoencephalitis
NLE	Necrotizing Leucoencephalitis
NME	Necrotizing Meningoencephalomyelitis
NR	Not Related
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PET	Positron Emission Tomography
PM	Post Mortem
PSV	Peak Systolic Velocity
QAR	Quiet Alert Responsive
RI	Restrictive Index
ROC	Receiver Operating Characteristics
RTA	Road Traffic Accident
SC	Subcutaneous
SE	Standard Error
SO-MUA	Spinal-Only Meningoencephalomyelitis of Unknown Aetiology
SRMA	Steroid Responsive Meningitis Arteritis
ST	Survival Time
STIR	Short Tau Inversion Recovery
T1W	T1-weighted
T1WI	T1-weighted Images
T2W	T2-weighted
T2WI	T2-weigthed Images
TNCC	Total Nucleated Cell Count
TE	Echo Time

TP	Total Protein
TR	Repetition Time
WBC	White Blood Cell

General Introduction

Adapted from: Cornelis I, Van Ham L, Gielen I, De Decker S, Bhatti S. Clinical presentation, diagnostic findings, prognostic factors, treatment and outcome in dogs diagnosed with meningoencephalomyelitis of unknown aetiology. *Submitted to The Veterinary Journal.*

Introduction

Idiopathic non-infectious meningoencephalomyelitis (NIME) encompasses a group of idiopathic, non-infectious central nervous system (CNS) disorders (Talarico and Schatzberg, 2010; Coates and Jeffery, 2014). This group includes several subtypes, including steroid responsive meningitis-arteritis (SRMA), eosinophilic meningoencephalitis (EME), and meningoencephalomyelitis of unknown aetiology (MUA). As SRMA (which affects the meninges only) and EME have fairly distinct diagnostic findings, the term MUA is introduced to cover the three specific subtypes of NIME that can only be confirmed based on histopathology, being granulomatous meningoencephalomyelitis (GME) and necrotizing encephalitis (NE) (including necrotizing meningoencephalomyelitis (NME) and necrotizing leucoencephalitis (NLE)) (Granger et al., 2010; Talarico and Schatzberg, 2010; Coates and Jeffery, 2014) (**Figure 1.1**).

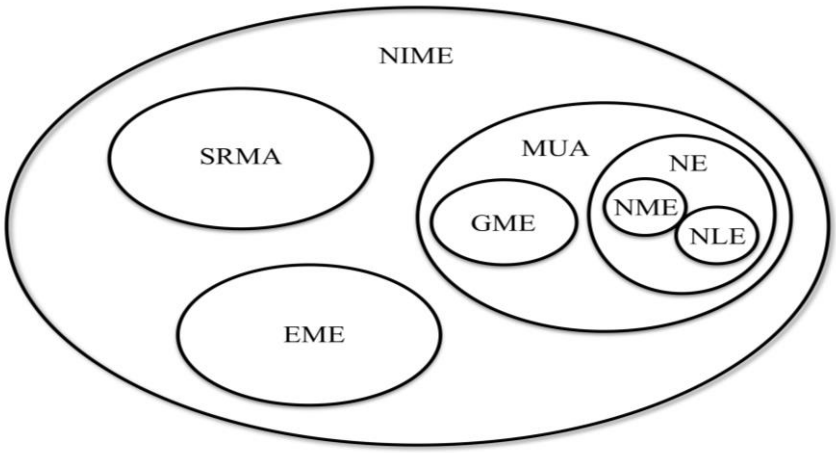

Figure 1.1*: Overview of the current classification of NIME.*

Overall, a clinical diagnosis of MUA can be achieved based on a combination of signalment, neurological examination results, magnetic

resonance imaging (MRI) findings and cerebrospinal fluid (CSF) analysis (Munana and Luttgen, 1998; Adamo et al., 2007; Granger et al., 2010; Talarico and Schatzberg, 2010; Coates and Jeffery, 2014), although these findings might vary substantially between patients (Wong et al., 2010).

This group of diseases offers both a diagnostic and therapeutic challenge to owners and veterinarians. As the condition is considered fatal without initiation of appropriate treatment (Munana and Luttgen, 1998; Granger et al., 2010), recent studies have evaluated different treatment modalities and potential prognostic factors.

Aetiology

The exact aetiology and pathophysiology of MUA are currently unknown and the most current theories were covered and discussed in a recent literature review (Coates and Jeffery, 2014). Although MUA has most likely a multifactorial pathogenesis, the combination of a genetic predisposition and factors triggering an excessive immunologic response are considered the two most important factors in the development of this disorder (Kipar et al., 1998; Talarico and Schatzberg, 2010; Flegel et al., 2011; Coates and Jeffery, 2014). Suspected triggering factors include environmental factors or infectious antigenic triggers that might activate autoreactive cells in the CNS, although no such agent has yet been identified (Schatzberg et al., 2005; Barbet et al., 2010; Greer et al., 2010; Barber et al., 2012). Combination of this information with the generally positive response to immunosuppressive treatment suggests that conditions comprising MUA are immune-mediated diseases (Wong et al., 2010), and the cornerstone of medical treatment is therefore considered immunosuppressive therapy (Kipar et al., 1998; Talarico and Schatzberg, 2010; Coates and Jeffery, 2014).

Clinical presentation

Middle-aged toy and terrier breeds are considered predisposed for GME (Munana and Luttgen, 1998; Adamo et al., 2007; Talarico and Schatzberg, 2010) whilst NE predominantly affects younger toy and small breed dogs including Pug, Yorkshire Terrier, Maltese, Chihuahua, Pekingese, Papillon, Shih Tzu, Coton de Tulear and Brussels Griffon (Talarico and Schatzberg, 2010; Cooper and others, 2014). However, it is stated that dogs of any breed and age can be affected (Granger et al., 2010; Coates and Jeffery, 2014).

Statistical analysis on 173 GME cases, 53 MUA cases and 69 NE cases revealed a significant difference in age distribution between dogs affected with GME and NE; dogs affected with NE were predominantly under 4 years old whereas the peak age for GME was 4-8 years (Granger et al., 2010). Historically, NME was described in Pug dogs with ages ranging from 6 months to 7 years (Cordy and Holliday, 1989), whilst dogs with a histopathological diagnosis of GME had ages ranging from 6 months to 12 years (Munana and Luttgen, 1998). In a series of 60 Pugs with NE (Levine et al., 2008), the median age was 18 months. In Pugs, fawn females were significantly more often diagnosed with NME compared to black males (Greer et al., 2009). Although female predominance is a widely held belief in GME (Cordy, 1979; Russo, 1979; Braund, 1985; Bailey, 1986; Sorjonen, 1990; Munana and Luttgen, 1998), no statistical difference in female:male ratio could be found in more recent studies (Talarico and Schatzberg, 2010; Granger et al., 2010).

Extraneural signs are rare, but pyrexia can occasionally accompany CNS inflammation (Talarico and Schatzberg, 2010). Common laboratory tests (complete blood count, biochemistry profile, urinalysis) are often within normal limits, however, results consistent with both inflammation and stress have been reported in dogs with GME (Thomas and Eger, 1989; Sorjonen, 1990; Tipold, 1995). Concurrent myocardial necrosis has been reported in two Pug dogs with NME, which was thought to be due to catecholamine release by the sympathetic nervous system (Bradley, 1991; Kobayashi et al., 1994).

On neurological examination, disease localisation was categorized as a) mainly forebrain, brainstem or multifocal for GME, b) focal (forebrain, brainstem) or multifocal in MUA, and c) mainly forebrain in NE (Granger et al., 2010; Talarico and Schatzberg, 2010; Coates and Jeffery, 2014). Eight percent of dogs diagnosed with GME were presented with neurological deficits suggestive of a myelopathy (Granger et al., 2010), that could be located anywhere along the spinal cord with clinical signs ranging from general proprioceptive ataxia to paresis or plegia, with spinal hyperesthesia as a common finding (Griffin et al., 2008; Wong et al., 2010).

Diagnostic findings

As previously stated, MUA is a clinical diagnosis that can be achieved based on a combination of signalment, neurological examination results, cross-sectional intracranial imaging findings and CSF analysis (Munana and Luttgen, 1998; Adamo et al., 2007; Talarico and Schatzberg, 2010; Coates and Jeffery, 2014). The study of Granger et al. (2010) systematically reviewed 457 published cases with NIME (including MUA, GME and NE) and formulated guidelines to recruit cases diagnosed with MUA in the absence of a histopathological diagnosis. The 4 following inclusion criteria have been formulated: 1) dogs older than 6 months of age, 2) multiple, single or diffuse intra-axial hyperintense lesions on T2-weighted (T2W) magnetic resonance images (MRI), 3) pleocytosis on CSF analysis with >50% of monocytes/lymphocytes, 4) ruling out infectious diseases commonly occurring in the specific geographic area (Granger et al., 2010). As stated previously, a definitive diagnosis can only be obtained by histopathological examination. The authors refer to a recently published review article on pathological and immunological features of GME and NME in dogs for further details (Uchida et al., 2016). The most important findings are summarized in **table 1.1.**

Both stereotactic computed tomography (CT) - guided brain biopsy procedures (Koblik et al., 1999) and free-hand biopsies through a mini-burr hole (Flegel et al., 2012) have been described in dogs with inflammatory CNS disease. Diagnostic accuracy ranged from 82% (n=17) (Flegel et al., 2012) to

100% (n=3) (Koblik et al., 1999) though results should be interpreted with caution due to the relative small sample sizes. Complications occurred in 12-29% of dogs, with associated signs being: transient epistaxis, transient exacerbation of neurological signs, obtundation progressing to coma, medically uncontrollable seizures, tetraparesis, hemiparesis, ataxia and loss of conscious proprioception (Koblik et al., 1999; Flegel et al., 2012). Although most of these signs resolved within 3-14 days, an indirect fatality rate of 6% was noted (Flegel et al., 2012).

Table 1.1. *Summary of pathological and immunological features of NME, NLE and GME (adapted from Coates and Jeffery, 2014; Uchida et al., 2016).*

Disease	Pathological features	Immunological features
NME	Acute NME (Pug with acute onset of seizures): absence of necrotic changes; diffuse leptomeningeal infiltration of lymphocytes, swelling of vascular endothelial cells in the superficial cerebral cortex Subacute or "typical" NME: multifocal, asymmetrical necrosis in the deep cerebral cortex consisting of perivascular infiltration of lymphocytes and macrophages, proliferation of reactive microglia around areas of necrosis Chronic NME: extensive (malacia) in cerebral cortex, nuclei of the thalamus, mesencephalon and cerebellum; moderate astrogliosis with proliferation of gemistocytic astrocytes (unique change in subacute and chronic NME).	GFAP1 astrocytes distributed widely over cerebrum; CD31 lymphocytes scattered in meninges, perivascular cuffs, and brain lesions but less compared with GME; MAC-3871 cells limited in NME but mainly in meninges and perivascular cuffs; lysozyme1 cells faint compared with GME; expression of IFN-g and CXCR3 highest in NME compared with NLE and GME. CD163 macrophages localized in active inflammatory lesions perivascular cuffs and brain parenchyma.

Disease	Pathological features	Immunological features
NLE	Malacic lesions in the cerebral white matter and thalamus; minimal lesions are occasionally found in the mesencephalon, cerebellum and brainstem; extensive necrosis with mild infiltration of myelin-laden macrophages in the white matter of the cerebrum and thalamus; mild to moderate mononuclear cell infiltration in meninges and perivascular areas of cerebral white matter.	Intralesional GFAP expression, CD3+ T cells dominate in perivascular cuffing and in diffuse histiocytic and lymphocytic infiltrates; rare B cells; MAC-387+ histiocytic cells were detected in lesions of Yorkshire terrier but few in French Bulldog; IgG deposits in white matter associated with inflammation; faint labeling IgM and IgA; CD163+ cells diffusely infiltrated the cerebral white matter.
GME	Cerebral white matter, cerebellum and brainstem are predominantly involved; perivascular cuffing of mononuclear cells (lymphocytes and macrophages).	CD3 lymphocytes in perivascular cuffs, parenchymal granulomas, and leptomeninges; CD43 and CD45R1 expression were low; expressions for B cells and plasma cells were low; strong MHC class II antigen expression observed in resting and activated T and B lymphocytes; MAC-3871 common; CD163 macrophages, epithelioid cells more frequent in perivascular cuffs than NME and NLE and in parenchymal lesions; CCR2 and highest in GME compared with NME and NLE; lysozyme1 histiocytes

Cross-sectional imaging

MRI has been reported to be 94.4% sensitive and 95.5% specific for detecting a brain lesion with similarly high performance for classifying neoplastic and inflammatory disease. On the contrary, MRI has been only 38.9% sensitive for classifying cerebrovascular disease. In general, high specificity but no sensitivity was retained for MR diagnosis of specific brain diseases (Wolff et al., 2012).

MR imaging is considered the most sensitive imaging modality for detecting intracranial lesions, but up to 7% (2/25 dogs, one diagnosed with GME and one with MUA) of scans showed no lesion on T2W images (T2WI) (Talarico and Schatzberg, 2010; Granger et al., 2010). Up to 14% (5/36 dogs, specific diagnosis not specified) of CT scans revealed no lesion (Granger et al., 2010). Overall, the sensitivity of imaging in identifying all inflammatory lesions suspected from the neurological examination remains quite low (<60%) (Granger et al, 2010). Additionally, MRI abnormalities were only seen in 76% of cases with inflammatory CSF findings in 1 study (Lamb et al, 2005). Although the use of cross-sectional imaging might aid in differentiating between the different types of idiopathic meningoencephalitides (Talarico and Schatzberg, 2010), no study is currently available looking into the use of MRI to differentiate between histopathologically confirmed cases of GME, NME and NLE.

In the literature, one study specifically focuses on the MRI findings in 11 dogs with histopathologically confirmed **GME** (Cherubini et al., 2006). The focal, multifocal or diffuse lesions were located in the forebrain, brainstem or cerebellum, and were hyperintense on T2W and fluid attenuating inversion recovery (FLAIR) images (**Figure 1.2**). Lesions were scattered throughout grey and white matter, showed variable intensities on T1-weighted images (T1WI) and variable degrees of contrast enhancement. Vasogenic oedema in the white matter was commonly present on T2-weighted images (T2WI), where meningeal enhancement was not commonly apparent and minimal if present (Cherubini et al., 2006; Talarico and Schatzberg, 2010; Coates and Jeffery,

2014). The lesion distribution (grey/white matter) was consistent with the histopathological findings (Cherubini et al., 2006).

The most common MRI abnormalities in dogs with **NME** include bilateral but asymmetrical, multifocal forebrain lesions (more severe lesions in parietal and occipital lobes have been described), hyperintense on T2W and FLAIR images, typically affecting the cortical grey and subcortical white matter with loss of grey/white matter demarcation and variable degrees of contrast enhancement of the parenchymal lesions on T1-weighted (T1W) post-contrast images (Flegel et al., 2008; Young et al., 2009; Talarico and Schatzberg, 2010) (**Figure 1.3**). However, cerebellar and brainstem lesions were additionally detected in 4/18 and 3/18 cases in one study, respectively (Young et al., 2009). Meningeal enhancement could be present, accompanied by mass effect and varying degrees of ventriculomegaly (Coates and Jeffery, 2014).

In **NLE**, multiple, bilateral but asymmetrical cerebral white matter and brainstem lesions have been detected (von Praun et al., 2006). These lesions were typically hyperintense on T2W and FLAIR images and often included multiple cystic areas of necrosis. Contrast enhancement of parenchymal lesions was minimal (Talarico and Schatzberg, 2010; Coates and Jeffery, 2014). There was lack of meningeal enhancement and mass effect, with varying degrees of ventriculomegaly (Coates and Jeffery, 2014) (**Figure 1.4**).

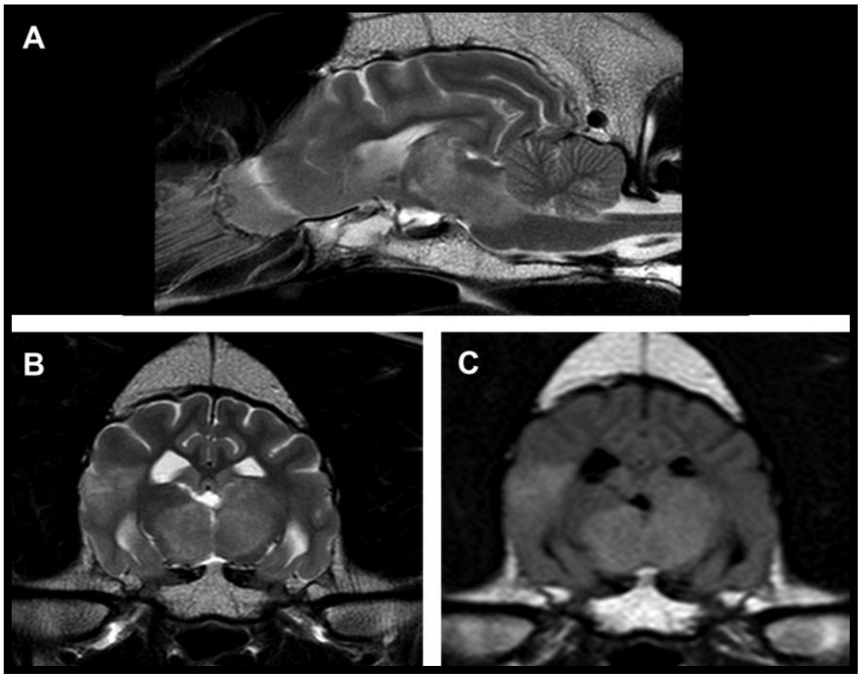

***Figure 1.2**: Mid sagittal (A) and transverse (B) T2W and transverse FLAIR image (C) at the level of the interthalamic adhesion in a 6-year-old female entire Golden Retriever with a histopathological diagnosis of GME. Note the diffuse hyperintensities on the T2W and FLAIR images affecting grey (both cortical and deep grey matter) and white matter involving forebrain (temporal lobe) and brainstem (Images courtesy of The Royal Veterinary College, University of London).*

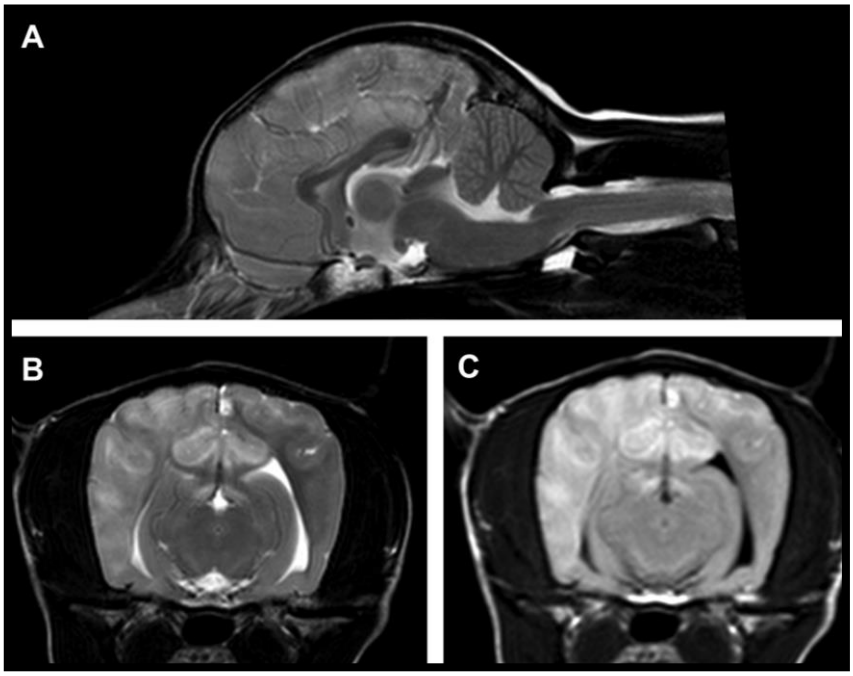

***Figure 1.3**: Mid sagittal (A) and transverse (B) T2W and transverse FLAIR image (C) at the level of the interthalamic adhesion in a 2-year-old female entire Maltese Terrier with a histopathological diagnosis of NME. Note the diffuse forebrain lesion affecting the cortical grey and subcortical white matter on the T2W and FLAIR images, involving the frontal, temporal and parietal lobes. Mass effect causing loss of cerebral sulci and occlusion of the right lateral ventricle can be observed. The deep cerebral grey matter, brainstem and cerebellum seem unaffected in the presented case (Images courtesy of The Royal Veterinary College, University of London).*

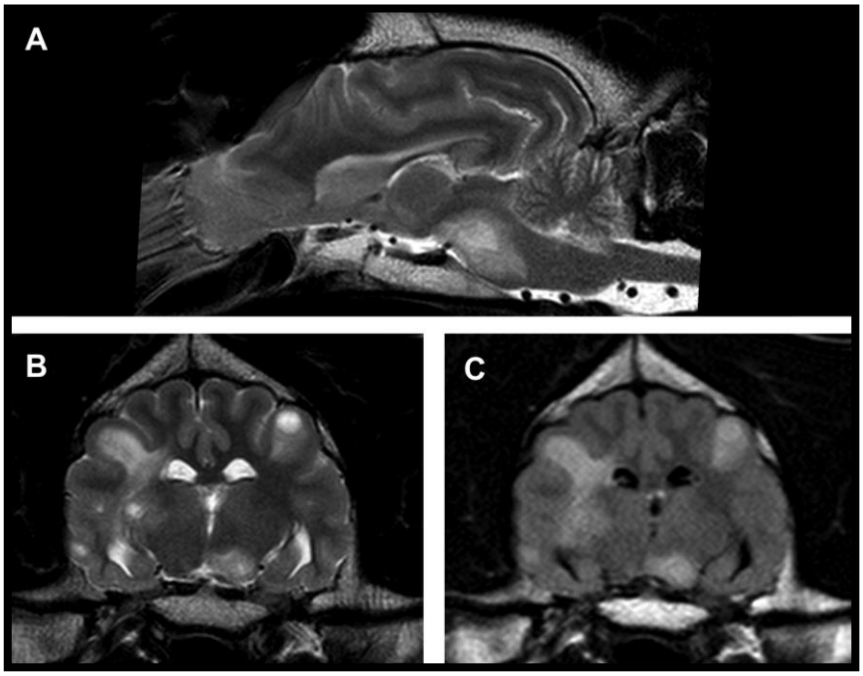

***Figure 1.4**: Mid sagittal (A) and transverse (B) T2W and transverse FLAIR image (C) at the level of the interthalamic adhesion in a 4-year-old male neutered Labrador Retriever with a histopathological diagnosis of NLE. Note the multiple lesions mainly affecting the cerebral white matter and the brainstem. Cystic areas were present throughout the forebrain white matter (Images courtesy of The Royal Veterinary College, University of London).*

Thirty-three dogs with MUA only involving the spinal cord have been reported, including 3 dogs with GME (Cherubini et al., 2006; Griffin et al., 2008; Wong et al., 2010). Imaging findings were available for 15 of these 33 cases, using different types of imaging modalities. Twelve dogs underwent myelography alone or CT-myelography, revealing no abnormalities in 11 dogs and a ventral extradural spinal cord compression in 1 dog (Wong et al., 2010). MRI was performed in 3 dogs, revealing no abnormalities in 1 dog, and multifocal poorly demarcated intramedullary T2W hyperintensities with variable contrast enhancement in 2 dogs (Cherubini et al., 2006; Wong et al., 2010).

Other imaging modalities, including positron emission tomography (PET) in NME, fluorodeoxyglucose PET (FDG-PET) and single voxel proton magnetic resonance spectroscopy (^{1}H MRS) in MUA, and transcranial sonographic findings in GME were investigated as diagnostic modalities (Eom et al., 2008; Kang et al., 2009; Carvalho et al., 2012; Carrera et al., 2016).

FDG-PET is a new imaging technique evaluating *in-vivo* tissue metabolism with the use of a metabolic tracer that acts as a glucose molecule. By this means, the tracer is transported into the tissue and trapped, which can afterwards be visualized. In both studies, a total of 5 dogs with NME, 1 dog with GME and 1 dog with MUA have been studied (Eom et al., 2008; Kang et al., 2009). Interestingly, all dogs with NME showed glucose hypometabolism (most likely attributed to the presence of malacia and necrosis), whereas glucose hypermetabolism was seen in GME (most likely due to strong granulomatous inflammatory reaction). In conclusion, the authors stated that further studies with larger sample sizes are necessary to confirm the associations (Eom et al., 2008; Kang et al., 2009).

^{1}H MRS is a non-invasive imaging diagnostic technique that provides specific biochemical information on numerous intracellular metabolites by measuring the signal that is emitted by proton nuclei because of their high magnetic sensitivity and presence in all tissues of the body. Long echo time sequences (typically >144 milliseconds) allow the determination of concentrations of N-acetyl aspartate (NAA), choline, creatinine and lactate, where short echo time sequences (typically <35 milliseconds) permit evaluation

of more metabolites, including myoinositol, glutamine, glutamate and lipids. This study has investigated 14 dogs with intracranial neoplasia and 15 dogs with MUA, and revealed that concentrations of NAA, creatinine and the glutamine-glutamate complex were reduced in the brains of dogs with neoplasia and MUA, whereas choline concentration was increased. Additionally, concentrations were significantly lower in dogs with neoplasia compared to MUA. A high concentration of taurine was also identified in the brains of 10/15 dogs with MUA (Carrera et al., 2016).

Transcranial B-mode Doppler sonography findings, performed through an intact skull, have been described in 11 dogs with GME (Carvalho et al., 2012). Diffusely decreased brain parenchyma echogenicity and hyperechoic focal lesions were observed in those dogs, and these findings were histopathologically comparable to congestion and inflammatory changes in the brain tissue and oval-shaped granulomas, respectively. Additionally, the restrictive index (RI), represented as peak systolic velocity (PSV) – (end diastolic velocity/PSV), was measured in 6 cerebral arteries (rostral, middle and caudal cerebral arteries of both left and right hemispheres). The RI was normal to high in all dogs, which can – according to the authors – be explained by intracranial pressure, which is normal to high in GME patients (Carvalho et al., 2012).

Cerebrospinal fluid analysis

Cerebrospinal fluid pleocytosis, defined as an increase in white blood cell (WBC) count (reference <5 WBC/mm^3), is one of the proposed diagnostic criteria for MUA (Granger et al., 2010). However, CSF analysis can be normal in 3-57% of dogs with MUA (Menaut et al., 2009; Granger et al., 2010), which is comparable to the results of a study in dogs with GME and NE, where CSF analysis revealed a normal cell count in 16% of dogs with GME and in 12.5% of dogs with NE (Granger et al., 2010). Albuminocytological dissociation has been found in cases with a normal cell count. Increased total protein (TP) concentration is a nonspecific indicator of CNS disease, typically caused by either blood-brain-barrier disruption or intrathecal immunoglobulin production

(Cordy, 1979; Tipold, 1995; Granger et al., 2010). Lymphocytes were the predominant cell type in 42% of GME cases and 71% of MUA cases, whereas monocytes and lymphocytes were found equally in NE; neutrophils were the predominant cell type in <10% of cases in each group (Granger et al., 2010). Reported total nucleated cell counts (TNCC) ranged from 50-900 WBC/mm^3 up to 11840 WBC/mm^3 in GME (Adamo et al., 2007; Granger et al., 2010) and from 0-6860 WBC/mm^3 in MUA (Granger et al., 2010). Reported CSF TP concentrations ranged from 40-400mg/dl (Adamo et al., 2007; Granger et al., 2010). In summary, most cases have a CSF mononuclear pleocytosis and as such a pleocytosis with >50% mononuclear cells is proposed as a diagnostic criterion for dogs with MUA (Granger et al., 2010; Coates and Jeffery, 2014).

Of the 33 reported dogs with meningomyelitis of unknown aetiology, CSF findings were only available for 1 dog (Cherubini et al., 2006). This revealed a pleocytosis, with a TNCC of 420 WBC/mm^3, and a TP concentration of 0.42g/l. The site of CSF collection was not mentioned (Cherubini et al., 2006).

Infectious disease testing

The exact aetiology of MUA remains unknown, but infectious antigenic triggers together with environmental factors have been described as potentials activators of autoreactive cells in the CNS, although nu such agent has yet been identified (Schatzberg et al., 2005; Barber et al., 2010; Greer et al., 2010; Barber et al., 2012). Schatzberg et al. (2005) investigated the presence of *Anaplasma Phagocytophylum, Ehrlichia canis, Toxoplasma gondii (T. gondii), Neospora caninum (N. caninum) and Cryptococcus neoformans* by serology, and presence of canine herpesvirus, adenovirus and canine parvovirus by polymerase chain reaction (PCR) in the brains of dogs diagnosed with NME (n=12), NLE (n=3) or GME (n=7), but all investigations returned negative. Swab et al. (2007) retrospectively investigated the brains of 53 dogs with non-suppurative meningoencephalitis for the presence of rabies virus, porcine herpesvirus, canine distempter virus (CDV), canine parvovirus, canine coronavirus, canine herpesvirus, canine adenovirus type I, Tick Borne

encephalitis, Borna disease virus, West Nile virus, canine parainfluenza virus, Encephalomyocarditis virus, prion proteins, *Listeria monocytogenes, Chlamydia* spp, *Mycoplasma* spp, and *Escherichia coli.* No infectious agent could be identified in 74% of cases, however, results should be interpreted with caution as obtaining a definitive diagnosis in those negative cases was not a specific aim of the study.

It has generally been advised to exclude regional infectious diseases (Granger et al., 2010), and this mostly includes to perform serology for *T. gondii* and *N. caninum* in dogs, and to perform PCR analysis for CDV. However, ante mortem diagnosis of *T. gondii* and *N. Caninum* can be challenging. For *T. gondii*, 3 diagnostic options are available, including 1) visualization of bradyzoites or tachyzoites in tissues, effusions, bronchoalveolar lavage fluids or CSF, 2) detection of *T. gondii* specific antibodies (IgG and IgM) in serum and 3) DNA amplification bij PCR on CSF (Lapin, 2014). Unfortunately, some shortcomings are present in that visualization is very uncommon, a failure to demonstrate increased IgM titers (titer above 1:64) or a fourfold or greater increase in IgG titers does not rule out disease; and PCR can reveal false negative results. Currently, the combination of IgM detection in CSF and a positive PCR analysis is the most accurate way to diagnose CNS toxoplasmosis (Lapin, 2014). Similar diagnostic criteria account for *N. caninum*: 1) visualization in tissue or CSF is very uncommon, 2) IgG antibody titers of > 1:800 are typically associated with clinical disease, whereas titers between 1:200 and 1:800 are considered undefined, and 3) DNA amplification by PCR analysis which returned positive in 4/5 dogs in one study, indicating that false negative results are possible (Lapin, 2014; Parzefall et al., 2014). Only sparse literature is available regarding prevalence of *T. gondii* and *N. caninum* in Belgium. On study stated that none of 2324 fecal samples examined (both dogs and cats) for *T. gondii* were positive (Vanparijs et al., 1991), and another study revealed that approximately 10% of Flemish dogs was seropositive for *N. caninum* (Barber et al., 1997). Currenlty, no studies are available on prevalence of CDV in Belgian dogs.

Although *T. gondii* is known to occasionally cause meningoencephalitis in dogs (Dewey, 2016), currently no MRI reports describing those findings are

available. On the contrary, some literature is available for cats, describing the presence of an intracranial granuloma that might resolve after appropriate treatment, consisting of surgical removal and/or antibiotic therapy (Pfohl and Dewey, 2005; Falzoni et al., 2008). *N. caninum* has previously been associated with necrotizing cerebellitis and cerebellar atrophy (Garosi et al., 2010) as well as with spinal cord changes, mesencephalic and metencephalic lesions, and with multifocal brain lesions on MR imaging in dogs (Parzefall et al., 2014). Three forms of CDV encephalitis are currently described, including acute CDV infection, CDV infection in mature dogs and "old dog encephalitis" (Dewey, 2016). Dogs with acute CDV infection are mostly very young (<1 year) and mainly present with forebrain signs, where histopathology reveals a polioencephalopathy. Mature dogs (>1 year) with CDV infection tend to develop inflammatory demyelinating white matter disease primarily affecting the brainstem, cerebellum and spinal cord (leucoencephalomyelopathy), whereas "old dog encephalitis" mainly affects older dogs (>5 years) and presents with signs of forebrain dysfunction (visual deficits, behavioral changes) (Dewey, 2016). Currently, MRI findings are only described in a cohort of 5 puppies, revealing hyperintense lesions and loss of contrast between grey and white matter on T2-weighted images in the cerebellum and/or brainstem. The majority of lesions were located in the temporal lobe of the cerebrum, so results should be interpreted with caution as all 5 dogs were presented with seizures (Bathen-Noethen et al., 2008).

Treatment

Although the criterion-referenced standard for a clinical trial is a randomized, placebo-controlled, double-blinded, prospective study, it is generally accepted that use of a placebo control treatment group is unethical because dogs with MUA have a poor outcome without treatment (Coates et al., 2007; Smith et al., 2009; Coates and Jeffery, 2014). Historically, different inclusion criteria have been used, and because in some studies immunomodulary medication was only initiated later, e.g. after results for infectious disease testing returned negative, treatment results and outcomes are difficult to compare (Adamo et al., 2007; Coates et al., 2007; Wong et al., 2010).

As previously stated, the exact aetiology and pathophysiology of MUA remains unknown, but the cornerstone of medical treatment is immunosuppressive therapy. Several treatment protocols using different immunomodulating drugs, resulting in different long-term survival times have been reported (Sisson et al. 1989; Gregory et al., 1998; Munana and Luttgen, 1998; Adamo and O'Brien, 2004; Gnirs, 2006; Zarfoss et al., 2006; Adamo et al., 2007; Coates et al., 2007; de Stefani et al., 2007; Feliu-Pascual et al., 2007; Uriarte et al., 2007; Jung et al., 2007; Menaut et al., 2008; Pakozdy et al., 2009; Smith et al., 2009; Granger et al., 2010; Kang et al., 2009; Wong et al., 2010; Flegel et al., 2011; Jung et al., 2012; Jung et al., 2013; Lowrie et al., 2013; Beckmann et al., 2015; Mercier and Barnes Heller, 2015; Barnoon et al., 2015; Lowrie et al., 2016). A comprehensive overview of all immunomodulatory therapies for MUA can be found in **table 1.2.**

***Table 1.2**. Summary of immunomodulatory drug therapies for MUA, including the reported number of dogs and the initial drug dosages. Abbreviations: PO = per os; IV = intravenous. (Adapted from: Coates and Jeffery, 2014).*

Drug	Number of dogs	Initial dosages	References
Azathioprine + prednisolone	40	2mg/kg PO q24h for 2 weeks, then decrease to 2mg/kg q48h for azathioprine	Wong et al., 2010
Ciclosporine	5	6-30mg/kg PO q24h	Adamo and O'Brien, 2004; Adamo et al., 2007
Ciclosporine + ketoconazole	3	5-12mg/kg PO q24h ciclosporine + 8mg/kg PO ketoconazole	Adamo et al., 2007
Ciclosporine + prednisolone	23	6-30mg/kg PO q24h ciclosporine	Adamo and O'Brien, 2004; Gnirs, 2006; Adamo et al., 2007; Jung et al., 2007; Pakozdy et al., 2009; Kang et al., 2009; Jung et al., 2012; Jung et al., 2013
Cyclophosphamide and vincristine + prednisolone	10	Cyclophosphamide: 50mg/m^2 PO q48h for 8 weeks, then every other week Vincristine: 0.5mg/m^2 IV, every 7 days for 8 weeks, then every 14 days	Smith et al., 2009
Cytosine arabinoside + prednisolone	158	50mg/m^2 SC, q12h for 2 consecutive days, then repeat every 3 weeks for 4 cycles IV infusion: 200 mg/m^2 over 8 hours	Zarfoss et al., 2006; de Stefani et al., 2007; Menaut et al., 2008; Smith et al., 2009; Lowrie et al., 2013; Lowrie et al., 2016

Drug	Number of dogs	Initial dosages	References
Leflunomide + prednisolone	5	1.5-4mg/kg PO q24h	Gregory et al., 1998
Lomustine + prednisolone	32	60mg/m^2 PO every 6 weeks	Uriarte et al., 2007; Flegel et al., 2011
Mycophenolate mofetil + prednisolone	30	10-20mg/kg PO q12h, reduce after 1 month to 5-10mg/kg q12h	Feliu-Pascual et al., 2007; Barnoon et al., 2015
Procarbazine + prednisolone	31	25-50mg/m^2 PO q24h	Coates et al., 2007
Prednisolone	78	1 to 2mg/kg PO q12h for 3–4 weeks; 0.5–1mg/kg q12h for 6 weeks; then 0.25–0.5mg/kg q12h for 3 weeks; then 0.25–0.5mg/kg q24h for 3 weeks; then 0.25–0.5mg/kg q48h indefinitely	Coates et al., 2007; Pakozdy et al., 2009; Granger et al., 2010; Flegel et al., 2011; Mercier and Barnes Heller, 2015

Overall, treatment effect is monitored by clinical response and resolution of neurologic deficits, and occasionally by repeated CSF analysis and MR imaging (Coates and Jeffrey 2014). In a small cohort of dogs, Lowrie et al. (2013) suggested that a combination of MR imaging and CSF analysis provided greater sensitivity for prediction of relapse than one modality alone. The authors therefore suggested that treatment should only be tapered once MR imaging and CSF analysis (including TP concentration) returned to normal (Lowrie et al., 2013).

Glucocorticosteroids

Treatment with glucocorticosteroids (mostly prednisolone) only is generally associated with shorter survival times (ST) compared to combination therapy with other immunosuppressive agents (Munana and Luttgen, 1998; Jung et al., 2007; Pakozdy et al., 2009; Granger et al., 2010; Flegel et al., 2011; Mercier and Barnes Heller, 2015). However, in a clinical setting, adding more expensive immunosuppressive therapies to the glucocorticoid protocol might be financially prohibitive.

Glucocorticosteroids bind to a cytosolic glucocorticosteroid receptor, which then moves to the nucleus, binds to DNA, and influences gene transcription. Cellular effects include stabilization of cell membranes, inhibition of phospholipase A2 with resultant inhibition of the cyclooxygenase and lipoxygenase pathways, decreased release of cytokines interleukin (IL) -1 and IL-6, and downregulation of Fc receptor expression on macrophages. The early effects of corticosteroids are believed to predominantly result from a rapid decrease in phagocytic activity of splenic and hepatic macrophages, whereas the long-term effects result primarily from suppression of cell-mediated immunity (suppression of T-helper cells) (Nelson and Couto, 2014). Most common adverse effects include polyuria, polydipsia, panting, muscle weakness, dermatological changes, predisposition to infections, and muscle atrophy. Glucocorticosteroids might cause insulin resistance, hyperglycaemia, vacuolar hepatopathy, and hypercoagulability (Nelson and Couto, 2014). Glucocorticosteroids are typically initiated at immunosuppressive doses, followed by tapering to a minimal effective dose while maintaining fair (or better) quality of life (Zarfoss et al., 2006).

In the literature, 78 dogs diagnosed with MUA and receiving sole prednisolone therapy have been reported, and survival data were available for all dogs (Coates et al., 2007; Pakozdy et al., 2009; Granger et al., 2010; Flegel et al., 2011; Mercier and Barnes Heller, 2015). Median survival times (MSTs) ranged from 28 – 357 days (43/78) (Granger et al., 2010), 91 - 329 days (19/78) (Flegel et al., 2011) and 602 days (16/78) (Mercier and Barnes Heller, 2015) in

dogs receiving dosages ranging from 0.5 – 30 mg/kg/day (Coates et al., 2007; Pakozdy et al., 2009; Granger et al., 2010; Flegel et al., 2011; Mercier and Barnes Heller, 2015).

Cytosine arabinoside

Six studies evaluated treatment with cytosine arabinoside as an adjunctive treatment option to prednisolone in dogs diagnosed with MUA, covering a total of 158 cases. Cytosine arabinoside can be administered either as a continuous rate infusion (CRI) (doses ranging from 100-300 mg/m2 over 8-24 hours) or as 4 subcutaneous (SC) injections of 50 mg/m^2 in 48 hours (200 mg/m^2 in 48h) (Zarfoss et al., 2006; de Stefani et al., 2007; Menaut et al., 2008; Smith et al., 2009; Lowrie et al., 2013; Lowrie et al., 2016). Crook et al. (2013) showed that CRI administration of cytosine arabinoside provided a steady state concentration over the time it was administered compared to a rapid absorption and elimination when administered subcutaneously. A recent clinical study revealed a significantly better 3-month-survival in dogs initially receiving a CRI of cytosine arabinoside compared to the SC route (Lowrie et al., 2016).

Cytosine arabinoside is a synthetic nucleoside analogue, which crosses the blood-brain-barrier, undergoes enzymatic activation, competes for incorporation into nucleic acids and then competitively inhibits DNA polymerase in mitotically active cells. Additionally, it causes topoisomerase dysfunction and prevents DNA repair; inhibits ribonucleotide reductase; inhibits membrane glycoprotein synthesis; and promotes leukemic cell differentiation in culture. All the effects are dependent on both cell cycle (S-phase) and rate of DNA synthesis (Zarfoss et al., 2006; Coates and Jeffery, 2014).

Side effects in dogs were dose dependent and mainly included myelosuppression and gastro-intestinal upset (Zarfoss et al., 2006). However, no dose-limiting toxicities were mentioned in most cases (Lowrie et al., Menaut et al., 2009; Smith et al., 2009). Transient post-treatment lethargy, dysphagia or limb tremors (3/10); mild coat and skin changes (increased shedding or alopecia, mild localised dermatitis) (4/10); and transient to intermittent pelvic

limb weakness (3/10) was noticed in one study (Zarfoss et al., 2006). Three case reports described three additional side effects of cytosine arabinoside therapy. One dog developed infiltrative lung disease 24h after the 4th cytosine arabinoside infusion (300mg/m2), where after the dog was euthanized (Hart and Waddell, 2016). The second dog developed anterior uveitis three weeks after the 5th cytosine arabinoside treatment (50mg/m2 q12h for 48h), which resolved after treatment with topical antibiotics, non-steroidal anti-inflammatory drugs, artificial tears and local atropine (Bianchi and Dodi, 2007). At last, development of severe calcinosis cutis and deep pyoderma at the cytosine arabinoside injection site was described in three dogs (Volk et al., 2012).

Reported MSTs with additional cytosine arabinoside ranged from 26 to 1063 days (n=69) (Zarfoss et al., 2006; de Stefani et al., 2007; Menaut et al., 2008; Smith et al., 2009; Lowrie et al., 2013; Lowrie et al., 2016).

Ciclosporine

Ciclosporine therapy has been described as sole treatment for MUA (n=5) (Adamo and O'Brien, 2004; Adamo et al., 2007), or as combination therapy with prednisolone (n=23) (Adamo and O'Brien, 2004; Gnirs, 2006; Adamo et al., 2007; Jung et al., 2007; Pakozdy et al., 2009; Kang et al., 2009; Jung et al., 2012; Jung et al., 2013), ketoconazole (n=3) (Adamo et al, 2007), or cytosine arabinoside and prednisolone (n=1) (Behr et al., 2009). Overall, 32 dogs have been reported receiving initial doses ranging from 3 – 15 mg/kg PO every 12 hours, resulting in MSTs ranging from 236 to 930 days (Adamo and O'Brien, 2004; Gnirs, 2006; Adamo et al., 2007; Jung et al., 2007; Pakozdy et al., 2009; Kang et al., 2009; Jung et al., 2013). One dog receiving combination therapy survived for 1096 days (Jung et al., 2012).

Ciclosporine is a fungal polypeptide that interferes with macrophage and monocyte activation by inhibiting the transcription of alfa-interferon. It suppresses T-cell mediated immune responses through inhibition of synthesis of IL-2 and other cytokines. Although ciclosporine is lipophilic, it has poor blood-brain-barrier permeability (Adamo and O'Brien, 2004; Gnirs, 2006; Adamo et al.,

2007). Because the blood-brain-barrier might be disrupted during inflammation in MUA, therapeutic ciclosporine concentrations may be present in affected areas of the CNS (Adamo et al., 2007). Commercial ciclosporine is available in 2 very different types of oral formulations. A vegetable-oil based preparation that caused marked intraindividual and interindividual variations in blood drug concentrations, and a microemulsified form that results in a more consistent and predictable absorption. Oral bioavailability of the microemulsion has improved by up to 50% compared with the oil-based formulation (Archer et al., 2014).

Reported side effects include mild hypertrichosis and transient lymphopenia (Adamo and O'Brien, 2004), vomiting during first 2 weeks of treatment (5/10) (Gnirs et al., 2006; Pakozdy et al., 2009) and severe gastro-intestinal adverse effects with life-threatening anaemia (1/7) (Pakozdy et al., 2009). A range of side effects has also been described in dogs receiving 5mg/kg q24h (the approved atopy dosage), additionally including anorexia, urinary tract infections, persistent otitis externa, gingival hyperplasia and lymphadenopathy (Archer et al., 2014).

Other immunosuppressive agents

Other immunosuppressive agents have been described in combination with prednisolone for treatment of MUA, including azathioprine (n=40) (Wong et al., 2010), procarbazine (n=31) (Coates et al., 2007), lomustine (n=32) (Uriarte et al., 2007; Flegel et al., 2011), vincristine and cyclophosphamide (n=10) (Smith et al., 2009), leflunomide (n=5) (Gregory et al., 1998), and mycophenolate mofetil (n=30) (Feliu-Pascual et al., 2007; Barnoon et al., 2015).

Following side effects were described in those studies: myelosuppression (19%) and haemorrhagic enteritis (15%) with procarbazine (Coates et al., 2007); leucopenia, severe thrombocytopenia and haemorrhagic gastro-enteritis with lomustine (Flegel et al., 2011); myelosuppression, haemorrhagic cystitis and pyometra with vincristine and cyclophosphamide (Smith et al., 2009); and haemorrhagic diarrhoea within the first 2 weeks of treatment with mycophenolate mofetil (Feliu-Pascual et al., 2007; Barnoon et

al., 2015). The side effects encountered with the combination of vincristine and cyclophosphamide were unacceptable to the author, excluding this protocol for further investigation (Smith et al., 2009). On treatment with azathioprine (n=40), major adverse events were infrequent and included poor coat or thin skin (13/40), urinary tract infection (3/40), vomiting (3/40), corneal ulcers (2/40), diabetes mellitus (2/40), renal failure, keratoconjunctivitis sicca, cruciate ligament rupture, hepatic mass, mammary gland adenoma, lymphoma, demodectic mange and septic arthritis of a single joint. However, many of the adverse effects, such as weight gain, poor coat, hypertriglyceridemia, thrombocytosis, and elevated liver enzyme activities, could have been associated with concurrent administration of corticosteroids (Wong et al., 2010).

MSTs were available for some studies, being 425 days for procarbazine (Coates et al., 2007), 150-740 days for lomustine (Uriarte et al., 2007; Flegel et al., 2011), 198 days for vincristine and cyclophosphamide (Smith et al., 2009), 250 days for mycophenolate mofetil (Barnoon et al., 2015), and 1834 days for azathioprine (Wong et al., 2010).

Radiation therapy

Three studies comprising 17 dogs examined the additional effect of radiation therapy (Sisson et al., 1989; Munana and Luttgen, 1998; Beckmann et al., 2015). This resulted in MSTs of 404-476 days, without occurrence of early or late radiotherapy reactions (Munana and Luttgen, 1998; Beckmann et al., 2015).

Prognostic factors

As MUA is generally considered a fatal disease (Munana and Luttgen, 1998), multiple studies attempted to identify prognostic factors for dogs diagnosed with MUA. Unfortunately, different studies revealed conflicting results, making the majority of findings inapplicable in a clinical setting.

Younger age at time of diagnosis was significantly associated with improved survival in dogs with MUA (Oliphant et al., 2016). Munana and Luttgen (1998) found significant longer survival times with focal versus multifocal neurological signs in dogs with GME. Additionally, dogs with focal forebrain signs had a significantly longer survival time compared to dogs with focal signs related to other areas of the CNS. Dogs with focal forebrain signs that underwent radiation therapy had a significantly longer survival time compared to dogs with focal forebrain signs that did not undergo radiation therapy (Munana and Luttgen, 1998). The finding of increased survival for dogs with focal neurological signs was, however, not repeated in more recent studies in dogs with MUA (Coates et al., 2007; Lowrie et al., 2013). Dogs presenting specifically with seizures or altered mentation had significantly shorter survival times (Bateman and Parent, 1999; Coates et al., 2007; Granger et al., 2010). Also, a significantly longer MST was recorded in dogs that were presented within 7 days of onset of clinical signs, compared to those presented after more than 7 days, suggesting that early diagnosis and treatment might influence survival time (Barnoon et al., 2015).

One study identified a lower CSF TNCC to be significantly associated with improved survival in dogs with MUA (Oliphant et al., 2016), whilst others found that neither CSF TNCC nor protein concentration had an effect on survival time in dogs with MUA (Coates et al., 2007). One study suggested that serial monitoring of CSF TNCC and protein concentrations is a sensitive indicator of successful treatment of inflammatory disease; however, clinical relapse was not evaluated statistically (Cizinauskas et al., 2000). The study of Lowrie et al. (2013) failed to demonstrate an association between normal CSF analysis and improved outcome, but did find an association between abnormal CSF analysis at three months and relapse or poor outcome in dogs with MUA (Lowrie et al., 2013). In the study of Mercier and Barnes Heller (2015) CSF analysis was repeated 1 month after diagnosis, and their results suggested that serial CSF analysis might be a valid tool for monitoring success or failure of treatment in dogs diagnosed with MUA and treated with glucocorticoid monotherapy.

Different findings on MR imaging were evaluated for their possible prognostic value, but so far midline brain shift (Oliphant et al., 2016) and contrast enhancement on T1WI and lesion burden (lesion volume compared to parenchymal volume) (Young et al., 2009) could not be associated with survival in dogs with MUA and Pug dogs with NME, respectively. On the contrary, mass effect, loss of cerebral sulci and foramen magnum herniation were all significantly associated with death in dogs with MUA, however the clinical prognostic power was low for those findings and none of them was predictive of long-term outcome (Lowrie et al., 2013; Lowrie et al., 2016). Resolution of MRI lesions three months after diagnosis was indicative of a good outcome (Lowrie et al., 2013).

Outcome

Approximately 15% of dogs with GME will die before being treated (Munana and Luttgen, 1998; Granger et al., 2010). Despite initiation of appropriate and aggressive immunosuppressive treatment, 56% of dogs in one study died or was euthanized because of MUA, and 33% of deceased dogs did so within 3 days after diagnosis (Lowrie et al., 2013). Levine et al. (2008) showed that dogs with NME that had received any form of treatment had a significantly longer mean ST than those that received no treatment. Nevertheless, most dogs with MUA or GME that die, do so within the first 3 months after diagnosis (Thomas and Eger, 1989; Smith et al., 2009; Lowrie et al., 2013). Eighteen/nineteen dogs (95%) survived for one month in one study (Smith et al., 2009), and only 1 of those dogs failed to survive for 1 year. Additionally, dogs that survived for 1 year often lived for a relatively long period beyond this, suggesting that animals alive after 1 month might have a relatively good chance of living several more years (Smith et al., 2009).

In one study, relapse was recorded in 65% of dogs with MUA within a median of 210 days following diagnosis (Lowrie et al., 2013). This study revealed that abnormal CSF analysis at three months was associated with higher risk of relapse, but the combination of MRI and CSF analysis provided a greater sensitivity for predicting relapse than one modality alone. Discontinuing

treatment before resolution of MRI lesions always resulted in relapse (Lowrie et al., 2013).

Follow-up information was available for 29 out of 33 dogs described with spinal MUA (Griffin et al., 2008; Wong et al., 2010). Overall, 17/33 dogs died or were euthanized because of MUA, and 9 dogs were alive at time of data capture (Griffin et al., 2008; Wong et al., 2010). No significant difference in ST could be identified between dogs with neurological signs of a myelopathy or an encephalopathy (Wong et al., 2010).

Conclusions

In the absence of an immediate histopathological diagnosis, the clinician should rely on previously established clinical diagnostic criteria used for the diagnosis of MUA. MRI is considered the gold standard for diagnosing intracranial inflammatory lesions, but studies looking into differentiation between GME, NME and NLE are currently unavailable. Immunosuppressive drugs are considered the cornerstone of treatment. Several studies have been performed regarding long-term outcome in MUA, but no information is available about short-term survival in those dogs. Further studies are therefore necessary to explore the underlying aetiology and pathophysiology of MUA, with optimisation of clinical diagnosis and treatment protocols, and to identify clinically reliable prognostic indicators.

Scientific Aims

MUA is a complex neurological disorder that causes diagnostic, therapeutic and prognostic challenges to both clinical neurologists and investigators. Currently, there is controversy and discussion about possible aetiologies, diagnostic criteria, different therapeutic options, prognostic factors and outcome in dogs diagnosed with MUA.

Therefore, the general aim of this thesis was to gain more insight in the diagnosis (including clinical presentation and cross-sectional imaging findings), treatment, possible prognostic factors and outcome of dogs with MUA.

The specific aims of this study were:

- To evaluate the clinical presentation, diagnostic findings and long-term outcome in **large dogs** diagnosed with MUA.
- To evaluate the clinical presentation, diagnostic findings and long-term outcome in dogs diagnosed with **MUA only affecting the spinal cord.**
- To compare the efficacy of three **sole prednisolone treatment** schedules in dogs with MUA, and to describe their associated long-term outcome.
- To compare the efficacy of sole prednisolone therapy and **combination therapy with ciclosporine** in dogs with MUA.
- To evaluate prognostic factors for **short-term outcome** in dogs diagnosed with MUA.

Research Studies

Part I

Clinical Presentation And Diagnostic Findings

Chapter 1

CLINICAL PRESENTATION, DIAGNOSTIC FINDINGS AND LONG-TERM SURVIVAL IN LARGE DOGS WITH MENINGOENCEPHALOMYELITIS OF UNKNOWN AETIOLOGY

Ine Cornelis[a], Holger A. Volk[b], Steven De Decker[b]

[a]*Small Animal Department, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium.*

[b]*Clinical Science and Services, The Royal Veterinary College, University of London, Hatfield, United Kingdom.*

Adapted from: Cornelis I, Volk HA, De Decker S. (2016). Clinical presentation, diagnostic findings and long-term survival in large breed dogs with meningoencephalomyelitis of unknown aetiology. *Veterinary Record* 179, 147.

Abstract

Although several studies indicate that MUA might affect every dog breed at every age, little is known about clinical presentation, diagnostic findings and long-term survival in large dogs. The aim of this study was therefore to compare the clinical presentation, diagnostic findings and long-term survival between large and small dogs diagnosed with MUA. One hundred and eleven dogs met the inclusion criteria. Twenty-eight (25%) dogs were considered large dogs, compared to 83 (75%) small dogs. Large dogs presented significantly more often with a decreased mentation. Age, gender, duration of clinical signs prior to diagnosis, presence of seizures or cluster seizures, variables on complete blood count and cerebrospinal fluid analysis, and all variables on MRI were not significantly different between small and large dogs. Median survival time was 281 and 106 days for the large and small dogs respectively, with no significant difference in survival curves for both groups. Although considered not typically affected by MUA, 25% of dogs included in this study were considered large dogs. Therefore, MUA should be included in the differential diagnosis for large dogs presenting with intracranial neurological signs. If diagnosed with MUA, large dogs also carried a guarded prognosis.

Introduction

As stated in the introduction of this thesis, middle-aged female toy and terrier breeds are considered predisposed to develop GME (Munana and Luttgen, 1998; Adamo et al., 2007; Talarico and Schatzberg, 2010). Necrotising encephalitis (including NME and NLE) predominantly affects toy and small dogs including Yorkshire Terrier, Maltese Terrier, French Bulldog, Shih Tzu, Lhasa Apso, Chihuahua, Pug, Pekingese, Papillon, Coton de Tulear and Brussels Griffon (Talarico and Schatzberg, 2010; Cooper et al., 2014). Although it is stated that dogs of any breed and age can be affected by MUA (Coates and Jeffery, 2014), literature regarding differences in clinical presentation, diagnostic findings and long-term survival between small and large dogs is currently unavailable. It is unknown whether the non-infectious inflammatory encephalopathies diagnosed in large dogs are just a variation on a common etiologic theme or represent a truly different aetiology compared to the various almost breed-specific encephalitides regularly diagnosed in small dogs. The aims of this study where therefore to describe the clinical presentation, diagnostic findings and long-term survival in large dogs diagnosed with MUA compared to small dogs. We hypothesized that no differences would be detected in clinical presentation, diagnostic findings and long-term survival between small and large diagnosed with MUA.

Materials and methods

Case selection

The electronic medical database of the Small Animal Referral Hospital, Royal Veterinary College, University of London, was searched between January 2006 and April 2015 for dogs diagnosed with " meningoencephalitis of unknown origin (MUO)", "MUA", "GME", "NME", "NLE", "inflammatory CNS disease", "non-

infectious meningoencephalitis" and the fully written versions of the above-mentioned abbreviations. Dogs were included based on the criteria used by Granger et al. (2010), if they had (1) complete medical records available, (2) a complete neurological examination performed leading to a focal or multifocal intracranial neuroanatomical localisation, (3) inflammatory CSF analysis, (4) MR imaging of the brain demonstrating single, multiple or diffuse intra-axial hyperintense lesions on T2WI, and (5) outcome data available through revision of medical records or contacting the referring veterinarian by email or telephone. Dogs were excluded if (1) the clinical records or imaging studies were incomplete or not available for review, (2) dogs were diagnosed with meningomyelitis without clinical signs of intracranial involvement, (3) no pleocytosis was found on CSF analysis with the exception of dogs with signs of raised intracranial pressure (ICP) on imaging studies, in which case CSF collection was not performed, and if (4) outcome data were unavailable. Dogs with histopathological confirmation of the disease only needed to fulfil inclusion criteria (1) and (5).

Dogs were divided in two groups based on their body weight: dogs <15kg, in this paper referred to as small dogs; and dogs >15kg, in this paper referred to as large dogs. For dogs with a body weight around 15kg, mean body weight for male and female dogs as reported on the Kennel Club website (http://www.thekennelclub.org.uk/services/public/breed/standard-find.aspx) were used to consider them small or large dogs. Information retrieved from the medical records included breed, age at diagnosis, gender, body weight, results of general physical and neurological examination and neuroanatomical localisation, duration of clinical signs prior to diagnosis, results of CBC and biochemistry profile, results of CSF analysis, and lactate concentration on venous blood gas analysis. Duration of clinical signs prior to diagnosis was classified as peracute (<2 days), acute (2–7 days) or chronic (>7 days). For dogs that had CSF analysis performed, site of collection (cisternal or lumbar), TNCC, TP concentration and nucleated cell differential count were recorded. Total nucleated cell count was considered normal if <5 WBC/mm^3. Total protein concentration was considered normal for a cisternal collection if <0.25g/l and for a lumbar collection if <0.4g/l. Possible neuroanatomical localisations included forebrain, brainstem or cerebellum. Dogs with vestibular signs

attributable to an intracranial lesion were diagnosed with central vestibular signs. If more than 2 of the above mentioned regions appeared to be affected on the neurological examination, dogs were given a multifocal neuroanatomical localisation, where dogs with only one region affected were given a focal neuroanatomical localisation. Magnetic resonance imaging was performed under general anaesthesia with a permanent 1.5T magnet (Intera, Philips Medical Systems, Eindhoven, the Netherlands) and all images were reviewed by a board certified neurologist (SDD) using Osirix DICOM viewer (Osirix Foundation, V.5.5.2 Geneva, Switzerland). The reviewer was blinded for signalment, results of the neurological examination and necropsy findings if available. Sequences could vary, but studies included a minimum of T2WI (TR (ms) / ET (ms), 3000/120), T1WI (TR/TE, 400/8) and FLAIR images of the entire brain in a sagittal, transverse and dorsal plane. The T1WI were acquired before and after IV administration of paramagnetic contrast medium (0.1mg/kg, gadoterate meglumine, Dotarem, Guerbet, Milton Keynes, UK). Variables recorded were lesion localisation and distribution, presence of parenchymal or meningeal contrast enhancement and presence of mass effect (brain herniation, midline shift, flattening of gyri/sulci).

Statistical analysis

Data analysis was performed with the aid of a standard statistical software package (Prism, Graphpad Software Inc, La Jolla, California, USA). A Mann-Whitney *U* test was used to compare age, duration of clinical signs prior to diagnosis, venous blood lactate levels, white blood cell (total, neutrophil and lymphocyte) count on complete blood count (CBC), TNCC and TP concentration in CSF between small and large dogs. A fisher's exact test was used to compare differences in gender, presence of seizures and cluster seizures, neuroanatomical localisation (mentation, forebrain, brainstem, central vestibular) and imaging findings (lesion localisation, meningeal or parenchymal contrast enhancement, mass effect, brain herniation, flattening gyri/sulci, rostral or caudal transtentorial herniation, foramen magnum herniation) between small and large dogs. Numeric variables were expressed as median and IQR. A false discovery rate (FDR) as used by Benjamini

et al. (2001) was applied to control for the increased risk of falsely significant results in the multiple comparisons. Values of $P<0.05$ were considered significant. Survival analysis was performed using both a Log-rank (Mantel-Cox) and Gehan-Breslow-Wilcoxin test, resulting in MST calculation and Kaplan-Meier survival curves comparing survival percentage in small and large dogs. Survival was defined as time from diagnosis to death or euthanasia, including whether this happened because of disease progression or due to unrelated causes, or time from diagnosis to data collection for dogs that were alive at time of data capture. Dogs that died because of unrelated causes and dogs that were still alive at time of data capture were censored for calculations.

Results

Signalment

Database research revealed 549 results. Dogs were excluded if they did not match the inclusion criteria, or if the combination of signalment, clinical presentation and imaging findings was more suggestive for another intracranial disorder (neoplasia, vascular lesion). Finally, 111 dogs were included in this study. These included 28 (25%) large and 83 (75%) small dogs. Large dogs represented were English Springer Spaniel (n=6), cross breed (n=5), Labrador Retriever (n=4), Golden Retriever (n=2), Akita (n=2), and one each of the following breeds: Border Collie, Boxer, Bernese Mountain dog, Curly-Coated Retriever, German Wirehaired pointer, Great Dane, Shar-Pei, Siberian Husky, Welsh Springer Spaniel. Compared to the general hospital population admitted between January 2006 and April 2015, English Springer Spaniels were not significantly overrepresented (P=0.196). Small dogs included West Highland White terrier (n=22), Chihuahua (n=8), Maltese terrier (n=8), Pug (n=8), French Bulldog (n=7), Cavalier King Charles Spaniel (n=6), crossbreed (n=6), Yorkshire terrier (n=5), Border terrier (n=2), Boston terrier (n=2), Pomeranian (n=2), Poodle (n=2) and one each of the following breeds: Bichon Frise, Welsh Corgi Cardigan, Lhasa Apso, Papillon and Sheltie.

Clinical presentation and diagnostic findings

Large dogs had a significant shorter duration of clinical signs prior to diagnosis (P=0.012), more often presented with decreased mentation (<0.0001) and less often with cranial nerve deficits (P=0.027), and were more often diagnosed with a brainstem lesion on MRI (P=0.039) compared to small dogs. However, when a FDR of 10% was applied, only decreased mentation was found to be significantly different between both groups. Gender, age at presentation, neuroanatomical localisation, presence of seizures and cluster seizures, lactate concentration on venous blood gas analysis, TNCC and TP concentration on CSF analysis and the remaining MRI findings (meningeal or parenchymal contrast enhancement, mass effect, brain herniation, flattening gyri/sulci, rostral or caudal transtentorial herniation, foramen magnum herniation) were not different between small and large dogs. All statistical results can be consulted in **table 2.1**, and a clinical summery regarding the large dogs can be consulted in **table 2.2**.

***Table 2.1**: Investigated variables in small and large dogs diagnosed with MUA. Values are numbers with their percentage, or a median with interquartile range between brackets. * = P-value below the FDR threshold and thus considered to be significant.*

Variable	Small dogs (n=83)	Large dogs (n=28)	P value/FDR
Signalment			
Age (months)	52 (6 – 146)	60 (10 – 120)	0.7373/0.0678
Male	44 (53%)	17 (61%)	0.0653/0.0143
Female	39 (47%)	11 (39%)	0.0653/0.0178
Duration of clinical signs prior to diagnosis (days)	8 (1-180)	5 (1 – 60)	0.0116/0.0071
Clinical signs			
Seizures	19 (23%)	10 (36%)	0.2164/0.0286
Cluster seizures	13 (16%)	7 (25%)	1.0000/0.0929
Neuroanatomical localisation			
Forebrain	58 (70%)	22 (79%)	0.4875/0.0464
Brainstem	53 (64%)	18 (64%)	1.0000/0.0964
Central vestibular	27 (33%)	4 (14%)	0.0904/0.0214
Abnormal mentation	26 (31%)	25 (89%)	<0.0001/0.0036*
Blood work			
White blood cells (.10^9/l)	10.6 (3.52 – 32.8)	9.8 (4.66 – 25.1)	0.5888/0.05
Neutrophils (.10^9/l)	7.5 (2.4 – 28.3)	7.1 (3.2 – 22.3)	0.4420/0.0429
Lymphocytes (.10^9/l)	1.3 (0.1 – 3.6)	1.2 (0.3 – 2.7)	0.3434/0.0393
Venous blood gas			
Lactate (mmol/l)	1.5 (0.6 – 3.6)	1.6 (0.4 – 10.4)	0.8711/0.0786

Variable	Small dogs (n=83)	Large dogs (n=28)	P value/FDR
CSF analysis			
TNCC (WBC/mm^3)	97 (1 – 2560)	48 (1 – 2220)	0.3360/0.03572
Total protein (mg/dl)	0.54 (0.11 – 3.51)	0.43 (0.1 – 8.47)	0.7872/0.0714
MRI findings			
Focal lesion	24 (29%)	7 (25%)	0.7163/0.0607
Multifocal lesion	57 (69%)	20 (71%)	0.7335/0.0643
Diffuse lesion	6 (7%)	2 (7%)	1.0000/0.1
Forebrain localisation	66 (80%)	23 (82%)	0.7035/0.0571
Brainstem localisation	41 (49%)	20 (71%)	0.0414/0.0107
Cerebellar localisation	16 (19%)	5 (18%)	0.8893/0.0821
Mass effect	53 (64%)	13 (46%)	0.1297/0.025
Brain herniation	34 (41%)	10 (36%)	0.6586/0.0536
Caudal transtentorial herniation	29 (35%)	10 (36%)	0.9097/0.0857
Foramen magnum herniation	23 (28%)	7 (25%)	0.8066/0.075
Midline shift	31 (37%)	7 (25%)	0.2534/0.321
Flattening gyri/sulci	38 (46%)	13 (46%)	0.9140/0.0893

***Table 2.2**: Overview of breed, age, TNCC in CSF, TP concentration in CSF, MRI findings, ST and post mortem (PM) necropsy findings for the large dogs included in this study. NP: not performed*.

Case	Breed	Age (months)	TNCC (WBC/mm^3)	TP (g/l)	MRI findings (lesion localisation)	ST (days)	PM diagnosis
1	Akita	36	2220	4.65	Multifocal lesions brainstem and spinal cord	2	NP
2	Bernese Mountain Dog	45	12	0.38	Multifocal lesions forebrain and brainstem	120	NP
3	Border Collie	70	300	2.47	Multifocal lesions forebrain and cerebellum	92	ALIVE
4	Boxer	68	NP	NP	Multifocal lesions forebrain, brainstem and cerebellum	1	GME
5	Cross Breed	85	23	0.26	Focal lesion forebrain	1580	ALIVE
6	Cross Breed	64	1255	1.31	Multifocal lesions forebrain, brainstem and spinal cord	5	NP
7	Cross Breed	23	555	1.92	Focal lesion brainstem	1	GME
8	Cross Breed	26	1090	8.47	Focal lesion brainstem	1095	ALIVE
9	Cross Breed	12	90	0.62	Multifocal lesions forebrain, brainstem and cerebellum	2708	ALIVE
10	Curly-Coated Retriever	63	80	0.78	Multifocal lesions forebrain, brainstem and cerebellum	2190	ALIVE

Case	Breed	Age (months)	TNCC (WBC/mm^3)	TP (g/l)	MRI findings (lesion localisation)	ST (days)	PM diagnosis
11	English Springer Spaniel	61	62	0.6	Multifocal lesions forebrain and brainstem	2950	ALIVE
12	English Springer Spaniel	60	17	0.75	Diffuse lesions forebrain	1	NP
13	English Springer Spaniel	47	12	0.16	Multifocal lesions brainstem	72	ALIVE
14	English Springer Spaniel	61	29	0.23	Multifocal lesions forebrain and brainstem	2039	ALIVE
15	English Springer Spaniel	39	173	0.68	Focal lesion brainstem	1187	ALIVE
16	English Springer Spaniel	78	NP	NP	Multifocal lesions forebrain, brainstem and cerebellum	700	ALIVE
17	German Wirehaired Pointer	10	12	0.23	Multifocal lesions forebrain and brainstem	975	ALIVE
18	Golden Retriever	75	1	0.21	Multifocal lesions forebrain and brainstem	1	GME
19	Golden Retriever	60	22	0.46	Multifocal lesions forebrain and brainstem	1856	ALIVE
20	Great Dane	60	40	0.1	Multifocal lesions forebrain	1	NP

Case	Breed	Age (months)	TNCC (WBC/mm^3)	TP (g/l)	MRI findings (lesion localisation)	ST (days)	PM diagnosis
21	Japanese Akita	20	51	0.41	Multifocal lesions forebrain and brainstem	1	NP
22	Labrador Retriever	120	8	0.1	Focal lesion forebrain	3	NME
23	Labrador Retriever	47	3	0.24	Multifocal lesions forebrain and brainstem	1	NLE
24	Labrador Retriever	27	NP	NP	Diffuse lesions forebrain	184	ALIVE
25	Labrador Retriever	94	30	0.43	Multifocal lesions forebrain and brainstem	730	ALIVE
26	Shar-Pei	36	NP	NP	Multifocal lesions forebrain	2129	ALIVE
27	Siberian Husky	30	68	0.35	Focal lesion forebrain	18	NP
28	Welsh Springer Spaniel	89	675	5.56	Multifocal lesions forebrain and brainstem	3	GME

Infectious disease testing was performed in 78 dogs, including serology for *Toxoplasma gondii* and *Neospora caninum* in 58 dogs (16 large dogs and 42 small dogs), and/or and PCR analysis for *Toxoplasma gondii*, *Neospora caninum* and Canine Distemper Virus in 73 dogs (13 large dogs and 41 small dogs). Overall, infectious disease testing was lacking in 9 large dogs from which 1 dog had complete necropsy performed and 4 dogs were still alive at time of data capture with survival times ranging from 184 – 2039 days. These 4 dogs were all treated with an immunosuppressive treatment protocol. The 4 remaining large dogs died within 20 days after diagnosis and breeds included English Springer Spaniel, crossbreed, Siberian Husky and Akita.

Post-mortem examination was performed in 14 dogs, 8 small and 6 large dogs. Results included GME (5 small dogs, 4 large dogs), NME (3 small dogs, 1 large dog) and NLE (1 large dog).

Outcome

All 111 dogs were initiated on immunosuppressive doses of glucocorticosteroids at time of diagnosis, combined with cytosine arabinoside in 66 (80%) small dogs and 18 (64%) large dogs. Most dogs on cytosine arabinoside therapy had regular (3-4 weekly) re-examinations at a dedicated cytosine arabinoside clinic. Overall, dogs that were alive at time of data capture, were dogs that received sole prednisolone therapy or combined prednisolone and cytosine arabinoside treatment.

At time of data capture, 11 (39%) large dogs and 27 (33%) small dogs were alive. Conversely, 17 (61%) large and 56 (67%) small dogs had died. Of the deceased dogs, 15 (88%) large dogs and 47 (84%) small dogs died or were euthanized because of disease progression, compared to 2 (12%) large dogs and 9 (16%) small dogs that died or were euthanized for unrelated causes. Overall, 54% of the large dogs and 57% of the small dogs died or were euthanized because of disease progression. The MST was 281 days and 106 days for the small and large dogs respectively. There was no significant

difference in survival curves between small and large dogs (P=0.664) (**Figure 2.1**).

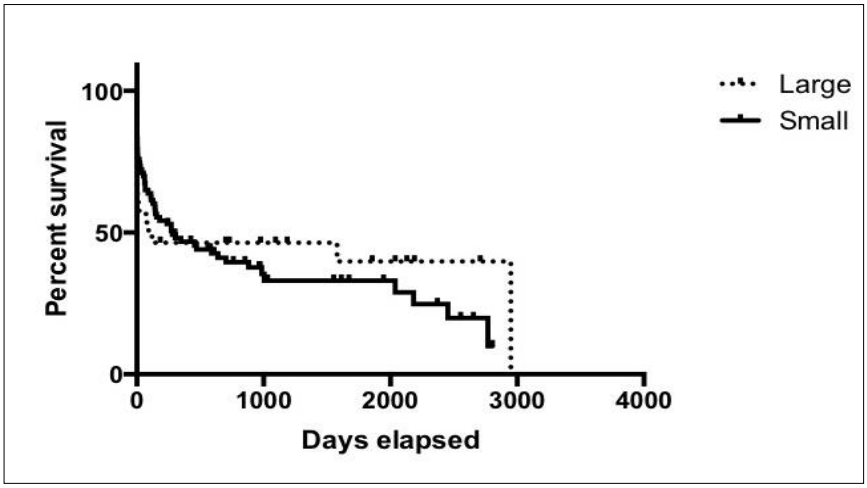

Figure 2.1*: Kaplan-Meier survival curve comparing the percentage of survival in small (full black line) and large (dotted line) dogs. Results were censored for dogs that were still alive at time of data capture (single little blocks).*

Discussion

This study evaluated the differences in clinical presentation, diagnostic findings and long-term survival between small and large dogs diagnosed with MUA. Large dogs were found to present significantly more often with a decreased mentation. No significant difference was seen in survival curves between small and large dogs. To the best of our knowledge, this is the first study describing clinical, diagnostic and outcome data in large dogs with MUA. Meningoencephalitis of unknown aetiology is generally considered a syndrome affecting small, toy and terrier breed dogs aged between approximately 3 and 7 years (Munana and Luttgen, 1998; Adamo et al., 2007; Talarico and Schatzberg, 2010; Coates and Jeffery, 2014). However, a total of 45 large dogs are present in the literature from 1998 to 2015 (Gregory et al., 1998; Munana and Luttgen, 1998; Cherubini et al., 2006; Fliegner et al., 2006; Zarfoss et al.,

2006; Coates et al., 2007; Pakozdy et al., 2009; Smith et al., 2009; Wong et al., 2010; Flegel et al., 2011; Lowrie et al., 2013; Estey et al., 2014; Barnoon et al., 2015; Beckmann et al., 2015; Mercier and Barnes Heller 2015). The distribution of the breeds in our study appears to be similar. Although the English Springer Spaniel was the most common large dog breed in our study, it was not significantly overrepresented compared to the general hospital population. The higher number of this breed in our study therefore likely reflects its popularity in the United Kingdom. MUA occurred, as expected, more often in small dogs, but still a quarter of dogs in the presented study were large dogs. Ignoring this population of dogs would underestimate the prevalence of MUA in the overall canine population. Therefore, MUA should be considered as a differential diagnosis in dogs other than small or toy breeds that have signs suggestive of inflammatory brain disease.

Large dogs were more likely to present with a decreased mentation compared to their small counterparts. Abnormal mentation in dogs can be caused by lesions in the forebrain (telencephalon and diencephalon) and/or brainstem (mesencephalon, metencephalon, myelencephalon) (Garosi, 2013). A lesion in the brainstem is considered a typical MRI finding in cases of GME and NLE, but is considered an uncommon finding in dogs with NME (Coates and Jeffery, 2014) although histopathological lesions have been identified in the brainstem of Chihuahua and Pug dogs with NME (Higgins et al., 2008; Park et al., 2012). In the presented study, both small and large dogs were histopathologically diagnosed with GME and NME, and 1 large dog was diagnosed with NLE, which has not yet been previously described. In literature, NLE is mainly affecting Yorkshire terriers and a French Bulldog (Schatzberg, 2005; Higginbotham et al., 2007; Timmann et al., 2007; Spitzbarth et al., 2010), which are all considered small dogs in this study. Necrotising meningoencephalitis has previously only been once described in a large dog; a 26kg Staffordshire Bull Terrier mix (Estey et al., 2014).

A limitation of the current study is the lack of histopathological confirmation in the majority of cases, which makes inclusion of other diseases than MUA possible (mainly cerebrovascular or neoplastic disease). Based on intracranial MRI, seven imaging abnormalities have been associated with

neoplastic brain disease in one study (Cherubini et al., 2005). These included the presence of a single lesion, regular lesion shape, presence of mass effect, dural contact, dural tail sign, lesions affecting adjacent bone and contrast enhancement (Cherubini et al., 2005). Another study demonstrated MRI to be 94.4% sensitive and 95.5% specific for detection of a brain lesion and for classifying neoplastic and inflammatory disease correctly, but was only 38.9% sensitive for classifying cerebrovascular disease (Wolff et al., 2012). For the presented study, 6 large dogs had a focal lesion on MRI of the brain, where inflammatory lesions are more typically associated with multifocal or diffuse lesions on intracranial imaging (Cherubini et al., 2006). Two of those lesions were histopathologically confirmed as GME or NME. Of the 4 remaining dogs, one dog (Siberian husky) had a focal lesion in the frontal lobe that showed presence of mass effect, parenchymal contrast enhancement and dural contact.

Although abnormalities on CSF analysis do not exclude neoplastic or vascular disease (Bohn et al., 2006), this dog had an increased TNCC (35 WBC/mm3) and TP concentration (0.35g/l) on cisternal CSF analysis. The dog was only 30 months old, and died acutely at home 18 days after diagnosis after initial improvement on immunosuppressive therapy. The second, third and fourth dog (2 cross breeds and an English Springer Spaniel) were 26 – 85 months old at time of diagnosis and had a focal lesion in the piriform lobe (crossbreed) or brainstem (cross breed and English Springer Spaniel). All three dogs had increased TNCC and TP concentrations on cisternal CSF analysis, and all were alive 1095 – 1580 days after diagnosis. All dogs had an acute onset of neurological signs, which might still be compatible with cerebrovascular disease, which might be supported by the long survival without treatment of those dogs. However, in cerebrovascular disease, contrast enhancement should only be visible after 7-10 days in dogs with ischaemic infarcts, and after 6 days to 6 weeks in dogs with haemorrhagic infarcts (Garosi, 2012). All dogs with a focal lesion had intracranial imaging within 5 days after onset of neurological signs, and all lesions showed parenchymal contrast enhancement, which was considered less typical for cerebrovascular disease. Neoplastic disease can however not be excluded in those cases, although all 4 dogs were still young at time of diagnosis (2-5y of age) where brain tumours are typically

affecting middle-aged to older (over 5 years) dogs and cats, with the majority being older than 9 years of age (Dickinson, 2014; Snyder et al., 2006).

Infectious disease testing was lacking in approximately 30% of both small and large dogs. The 9 large dogs that were not tested had focal (n=2), multifocal (n=5) or diffuse forebrain (n=2) lesions on MR imaging. Necrotising cerebellitis and cerebellar atrophy (Garosi et al., 2010) as well as multifocal brain involvement (Parzefall et al., 2014) have been described in association with *Neospora caninum* infection in dogs. In the study of Parzefall et al., (2014), mild cerebellar atrophy was additionally seen in 3 of 4 dogs. In the presented study, 11 large dogs died within 20 days after diagnosis and after immunosuppressive treatment. Necropsy confirming GME, NLE or NME was performed in 6/11 dogs. Of the 5 remaining dogs, all but 1 (Siberian Husky) had multifocal or diffuse forebrain lesions without cerebellar involvement, and showed no signs of perilesional edema on FLAIR images as described by Parzefall et al., (2014). Magnetic resonance imaging features of *Toxoplasma gondii* encephalitis have not yet been described for dogs, but it is known to cause focal granuloma formation both in the forebrain (Pfohl et al., 2005; Falzone et al., 2008) and the spinal cord (Alves et al., 2011) of cats. The 4 dogs with multifocal or diffuse brain lesions all had an increased TNCC with a mononuclear pleocytosis but without signs of presence of lymphoblastic cells. No further diagnostic investigations for lymphoma were performed. Gliomatosis cerebri is a central nervous system neoplasia that is mainly affecting brachycephalic breeds (Boxer, Boston Terrier, English Bulldog, Bull Mastiff) and MRI mainly reveals single lesions in thalamus and/or brainstem, although the cerebrum can be involved and sometimes no lesions are visible (Bentley et al., 2014). This differential diagnosis seems less likely in these 4 dogs because of their breeds and the presence of multifocal or diffuse lesions, but necropsy confirmation is lacking in those cases. Overall, pitfalls of this study are its retrospective character, the lack of histopathological confirmation and infectious disease testing in a number of cases. In contrary, MUA is considered a clinical and imaging diagnosis in cases were definitive brain histology is lacking.

Conclusions

Twenty-five per cent of dogs diagnosed with MUA in this study were considered large dogs. Ignoring this population of dogs would underestimate the prevalence of MUA in the overall canine population. Large dogs presented significantly more often with a decreased mentation compared to small dogs. The MST was not significantly different between small and large dogs, 281 and 106 days respectively, leading to a guarded prognosis for all dogs diagnosed with MUA and receiving immunosuppressive treatment. In conclusion, MUA should be considered as a differential diagnosis in dogs other than small or toy breeds that have signs suggestive of inflammatory brain disease.

Chapter 2

CLINICAL PRESENTATION, DIAGNOSTIC FINDINGS AND OUTCOME IN DOGS WITH SPINAL-ONLY MENINGOENCEPHALOMYELITIS OF UNKNOWN AETIOLOGY

Ine Cornelis[a], Holger A. Volk[b], Luc Van Ham[a], Steven De Decker[b]

[a]*Small Animal Department, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium.*
[b]*Clinical Science and Services, The Royal Veterinary College, University of London, Hatfield, United Kingdom.*

Adapted from: Cornelis I, Volk HA, Van Ham L, De Decker S. (2017). Clinical presentation, diagnostic findings and outcome in dogs diagnosed with spinal-only meningoencephalomyelitis of unknown aetiology. *Journal of Small Animal Practice* 58, 174-182.

Abstract

Although difficult to clinically diagnose, spinal-only meningoencephalomyelitis of unknown aetiology (SO-MUA) is an important differential diagnosis for dogs presenting with signs of spinal cord dysfunction. The aim of this study was to evaluate clinical presentation, diagnostic findings and long-term outcome for dogs clinically diagnosed with SO-MUA.

Twenty-one dogs were included in the study. The majority of dogs presented with an acute (43%) or chronic (52%) onset of neurological signs. Ambulatory paresis was the most common neurological presentation (67%). Neurological examination most commonly revealed a T3-L3 myelopathy, and spinal hyperaesthesia was a common finding (71%). A spinal cord lesion was visible in 90% of cases on MR imaging. Lesions were typically extensive, ill-defined, hyperintense on T2WI and isointense on T1W images (T1WI). Eighteen lesions (86%) showed parenchymal contrast enhancement and 17 lesions (81%) showed contrast enhancement of overlying meninges. All dogs were treated with immunosuppressive doses of glucocorticosteroids, sometimes combined with cytosine arabinoside. At time of data capture, 10/21 dogs (48%) had died or been euthanized because of SO-MUA. Overall median survival time was 669 days.

SO-MUA should be considered in the differential diagnosis of dogs presenting with an acute or chronic, progressive, and potentially painful myelopathy. MRI features can possible help to distinguish presumptive SO-MUA from other more common spinal diseases. Overall, long-term survival is guarded as approximately 50% of dogs will die or be euthanized because of SO-MUA regardless of immunosuppressive treatment.

Introduction

Pure myelitis (inflammation of spinal cord parenchyma) or meningomyelitis (inflammation of spinal cord parenchyma and surrounding meninges) are rare diseases in small animals and occur commonly in combination with inflammatory brain disease (Tipold and Stein, 2010). Viruses (canine distemper virus, feline coronavirus), bacteria (*Staphylococcus* spp., *Streptococcus* spp., *Pasteurella,* coliforms*, Actinomyces, Nocardia* spp.), fungi (*Cryptococcus, Coccidioides* spp., *Blastomyces, Histoplasma*), rickettsiae (*Ehrlichia, Rickettsia*, Rocky Mountain spotted fever), protozoa (*Toxoplasma gondii, Neospora caninum*), parasites (*Dirofilaria immitis, Cuterebra, Angiostrongylus vasorum*) and algae (*Prototheca wickerhamii, Prototheca zopfii*) are known causes of meningomyelitis in dogs and cats, with or without concurrent intracranial signs (Dewey and Da Costa, 2016; Csebi et al., 2010; Parry et al., 2009; Griffin et al., 2008). Apart from infectious causes, non-infectious meningomyelitis including GME, pyogranulomatous meningoencephalomyelitis and SRMA are described (Dewey 2016; Parry et al. 2009; Griffin et al. 2008; Meric 1988). In agreement with the terminology for MUA, dogs clinically diagnosed with non-infectious inflammatory myelitis that did not have positive infectious disease testing, that were not classified as SRMA or eosinophilic meningomyelitis, and that were not histopathologically confirmed, were named SO-MUA. A clinical diagnosis of SO-MUA is typically made by a combination of clinical presentation, imaging of the vertebral column, and results of CSF analysis (Griffin et al., 2008).

Currently, only one previous study has focused specifically on the clinical presentation, diagnostic findings, and outcome in dogs with meningomyelitis caused by a variety of underlying aetiologies (Griffin et al., 2008). Twenty-eight cases were included, of which 15 dogs were diagnosed with SO-MUA. Clinical signs reflected the affected spinal cord segments, and younger dogs, toy breeds, and hound breeds were suggested to be predisposed for meningomyelitis. Although results of myelography, CT, and CT-myelography have been reported, little is known about MRI findings in dogs with SO-MUA. The aims of this study were therefore to describe the signalment,

clinical presentation, diagnostic findings, including results of MRI, and long-term survival in dogs diagnosed with presumptive SO-MUA without concurrent clinical signs of intracranial involvement.

Materials and methods

Case selection

The electronic medical database of the Small Animal Referral Hospital, Royal Veterinary College, University of London, was searched between March 2006 and February 2015 for dogs diagnosed with “MUA”, “MUO”, “GME”, “myelitis”, and “inflammatory spinal cord disease”. Dogs were included based on the criteria used by Granger et al. (2010), if they had (1) complete medical records available, (2) a complete neurological examination performed leading to a spinal cord localisation, (3) inflammatory CSF analysis, (4) MRI of the spinal cord, and if (5) long-term follow-up information was available through revision of medical records or through contacting the referring veterinarian by telephone. Dogs were excluded if (1) the clinical records or imaging studies were incomplete or not available for review, (2) dogs showed clinical or neurological signs of intracranial involvement at time of presentation, (3) they had a peracute onset of clinical signs that were not progressive after 12-24 hours, (4) they had signs of extradural or intradural/extramedullary spinal cord compression on MRI and if (5) they had positive infectious disease titres or if clinical presentation, CSF analysis or necropsy findings were suggestive of SRMA or eosinophilic meningoencephalomyelitis (>10% eosinophils in CSF) (Dewey and Da Costa, 2016). Typical clinical presentation for SRMA was considered to be a dog less than 2 years of age of a typical dog breed (Boxer, Beagle, Bernese Mountain dog, Nova Scotia Duck Tolling Retriever, Golden Retriever, German Shorthaired Pointed) presenting with pyrexia and cervical hyperesthesia. CSF analysis in SRMA is typically revealing a predominantly neutrophilic pleocytosis (Dewey and Da Costa, 2016). Dogs with histopathological confirmation of the disease (GME or NME) only needed to fulfil inclusion criteria (1) and (5). Information retrieved from the medical records included breed, gender, age at

diagnosis, body weight, results of neurological examination including neuroanatomical localisation, duration of clinical signs prior to diagnosis, results of CBC and biochemistry profile, results of CSF analysis, treatment received, and outcome. For dogs that had CSF analysis performed, site of collection (cisternal or lumbar), TNCC, TP and cytological differentiation were recorded. Total nucleated cell count was considered normal if <5 cells/mm^3. Total protein concentration was considered normal for a cisternal collection if < 0.25g/l and for a lumbar collection if <0.4g/l. Duration of clinical signs prior to diagnosis (days) was classified as peracute (<2 days), acute (2–7 days) or chronic (>7 days).

Neurological assessment

The neurological status was classified from 0 to 5 according to the clinical examination (adapted from Scott et al., 1997): grade 0 = neurologically normal; grade 1 = spinal hyperesthesia without neurological deficits; grade 2 = ataxia, ambulatory para- or tetraparesis; grade 3 = non- ambulatory para- or tetraparesis; grade 4 = para- or tetraplegia with or with- out bladder control, and intact deep pain sensation; grade 5 = para- or tetraplegia, urine retention or overflow, and deep pain sensation loss.

Possible neuroanatomical localisations included C1-C5, C6-T2, T3-L3 or L4-S3 spinal cord segments. Dogs were diagnosed with a focal lesion if only one spinal cord segment was affected, and with a multifocal lesion if more than one spinal cord segment appeared to be affected on the neurological examination.

Magnetic resonance imaging

Magnetic resonance imaging was performed under general anaesthesia with a permanent 1.5T magnet (Intera, Philips Medical Systems, Eindhoven, the Netherlands) and all images were reviewed by the same author (IC) using Osirix Dicom viewer (Osirix Foundation, V.5.5.2 Geneva, Switzerland). Sequences could vary, but studies included a minimum of T2W

(repetition time (ms) (TR)/echo time (ms) (TE), 3000/120) and T1W (TR/TE, 400/8) images of the affected spinal cord region in a sagittal and transverse plane. The T1W images were acquired before and after IV administration of paramagnetic contrast medium (0.1mg/kg, gadoterate meglumine, Dotarem, Guerbet). If MR images of the brain were present, they were reviewed concurrently. Variables recorded were lesion intensity on T2WI and T1WI, lesion localisation and distribution, lesion length and presence of parenchymal and/or meningeal contrast enhancement. Lesion length was measured using Osirix Dicom viewer, and performed on sagittal T2WI for dogs that had focal lesions. Lesion length was measured twice, and the mean value between both was used. To compensate for differences in body size, values were corrected towards length of vertebral body of C6 (for cervical lesions) or L2 (for thoracolumbar lesions). Vertebral body length was measured on T1W sagittal images.

Treatment and follow-up

For all dogs, the specific treatment protocol was recorded. During hospitalisation, all dogs underwent daily at least one general physical and complete neurological examination by a board-certified neurologist or neurology resident. The results of the neurological examination as well as response to treatment (improvement, deterioration or static status) were systematically recorded on the kennel sheets. Follow-up information during hospitalisation was collected from the medical records, and afterwards through medical records of re-examination visits or telephone contact with the referring veterinarian. A successful outcome was defined as the dog being ambulatory, faecal and urinary continent and, according to the owners, without signs of overt spinal hyperaesthesia. An unsuccessful outcome was defined as (1) deterioration in neurological status by one or more grades after diagnosis and treatment, or (2) if the dog was not independently ambulatory, possibly with previously non-existing or worsening faecal and/or urinary incontinence, or was experiencing spinal hyperaesthesia as defined by the owner.

Statistical analysis

Data analysis was performed with the aid of a standard statistical software package (Prism, Graphpad Software Inc). Regarding outcome, a Mann-Whitney U test was used to evaluate the effect of relative lesion length on long-term outcome. A fisher's exact test was used to evaluate the effect of pain, presence of lymphopenia and additional administration of cytosine arabinoside on outcome. Numeric variables were expressed as median and interquartile ranges (IQR). Values of $P<0.05$ were considered significant. Survival analysis was performed using both a Log-rank (Mantel-Cox) and Gehan-Breslow-Wilcoxin test, resulting in MST calculation and a Kaplan-Meier survival curve. Survival was defined as time from diagnosis to death or euthanasia, including whether this happened because of disease progression or due to unrelated causes, or time from diagnosis to data collection for dogs that were alive at time of data capture. Dogs that died because of unrelated causes and dogs that were still alive at time of data capture were censored for survival analysis.

Results

Signalment

Twenty-one dogs were included in the study. Represented breeds included French Bulldog (n=2), Jack Russell Terrier (n=2), Lhasa Apso (n=2) and one each of Akita, Bearded Collie, Boxer, Bull Mastiff, Chihuahua, cross breed, English Springer Spaniel, Giant Schnauzer, Labrador Retriever, Maltese Terrier, Rhodesian Ridgeback, Rottweiler, Shih Tzu, West Highland White Terrier and Yorkshire Terrier. Overall, median age at presentation was 56 months (10 – 128 months). Thirteen dogs (62%) were male and 8 dogs (38%) were female. Compared to the general hospital population between March 2006 and February 2015, there was no significant difference in sex distribution in the group of dogs with SO-MUA ($P=0.075$). Median duration of clinical signs prior to diagnosis was 8 days (ranging from 1-90 days). One dog (5%) presented with

peracute, 9 dogs (43%) with acute and 11 dogs (52%) with a chronic onset of neurological signs.

Neurological examination

Thirteen (62%) and 8 (38%) dogs were diagnosed with a focal and multifocal spinal lesion on neurological examination, respectively. Regarding dogs with focal spinal lesions (n=13), 3 dogs were diagnosed with a lesion affecting the C1-C5 spinal cord segments, 2 dogs with a lesion affecting the C6-T2 spinal cord segments, 6 dogs with a lesion affecting the T3-L3 spinal cord segments and 2 dogs with a lesion affecting the L4-S3 spinal cord segments. At time of diagnosis, no dogs presented as grade 0; 2 dogs (10%) were grade 1; 14 dogs (67%) grade 2; and 5 dogs (24%) grade 3. No dogs were found to have paraplegia or tetraplegia at time of presentation. Pain on direct spinal palpation was present in 15 (71%) dogs. Urinary retention was seen in 2 dogs (10%), and a combination of urinary and faecal incontinence was noticed in 2 dogs (10%). One dog (5%) developed seizures 669 days after diagnosis of SO-MUA. An overview of the clinical and diagnostic findings of the 21 included dogs can be consulted in **table 3.1**.

***Table 3.1**: Clinical details of the 21 dogs diagnosed with SO-MUA. Dogs marked with * had additional intracranial abnormalities detected on MR imaging. FE = female entire, FN = female neutered, ME = male entire, MN = male neutered.*

Case	Breed	Gender	Age (months)	Clinical presentation	Neuroanatomical localisation	Spinal hyperaesthesia	CSF TNCC (WBC/mm^3)	MRI lesion
1	Akita	FE	36	Non ambulatory paraparesis	Multifocal	Yes	1740	Focal
2	Rottweiler	ME	123	Ataxia	T3-L3	No	209	Focal
3	Bull Mastiff	ME	42	Ambulatory paraparesis	T3-L3	Yes	6	No lesion visible
4	Labrador Retriever	MN	105	Ambulatory paraparesis	L4-S3	Yes	123	Focal
5	JRT	MN	89	Ambulatory paraparesis	T3-L3	No	200	Focal
6	Lhasa Apso	FE	48	Ambulatory tetraparesis	C1-C5	Yes	900	Focal
7	Shih Tzu	MN	50	Ambulatory tetraparesis	C6-T2	Yes	5	Focal
8	Giant Schnauzer	ME	32	Non ambulatory paraparesis	Multifocal	No	1345	Focal

Case	Breed	Gender	Age (months)	Clinical presentation	Neuroanatomical localisation	Spinal hyperaesthesia	CSF TNCC (WBC/mm^3)	MRI lesion
9	Yorkshire Terrier	FN	36	Ambulatory tetraparesis	C1-C5	Yes	7	Focal
10	English Springer Spaniel	ME	85	Ataxia	Multifocal	No	455	Focal
11	Rhodesian Ridgeback	FE	123	Normal gait	C1-C5	Yes	89	Focal*
12	Bearded Collie	MN	136	Ambulatory paraparesis	Multifocal	Yes	162	No lesion visible
13	Boxer	ME	26	Normal gait	Multifocal	Yes	6000	Focal
14	Lhasa Apso	MN	128	Ambulatory paraparesis	L4-S3	Yes	1540	Multifocal
15	Chihuahua	ME	19	Ataxia	T3-L3	Yes	9	Multifocal
16	Cross Breed	FN	83	Ambulatory paraparesis	Multifocal	No	1230	Multifocal
17	French Bulldog	ME	13	Ambulatory paraparesis	T3-L3	No	250	Multifocal
18	Maltese Terrier	FN	104	Ataxia	Multifocal	Yes	95	Focal

Case	Breed	Gender	Age (months)	Clinical presentation	Neuroanatomical localisation	Spinal hyperaesthesia	CSF TNCC (WBC/mm^3)	MRI lesion
19	Jack Russell Terrier	FN	56	Non ambulatory tetraparesis	C6-T2	Yes	2690	Focal*
20	French Bulldog	ME	10	Non ambulatory paraparesis	T3-L3	Yes	43	Focal
21	West Highland White Terrier	FE	103	Non ambulatory tetraparesis	Multifocal	Yes	1980	Multifocal

Diagnostic findings

As required by the inclusion criteria, CSF collection revealed a pleocytosis in all cases. Overall, median TNCC was 209 WBC/mm^3 (ranging from 6 – 6000 WBC/mm^3). Total protein measurement was performed in all but 3 CSF samples, and was above reference values in 17/18 dogs (94%). The median TP concentration was 1.67g/l (ranging from 0.21-16.3g/l). Complete blood count and serum biochemistry results were available in 16 dogs (76%). Leucocytosis was only present in 2 dogs (10%) and lymphopenia was present in 6 dogs (29%). Infectious disease testing based on serology and/or PCR on CSF for Canine Distemper Virus, *Toxoplasma gondii*, and *Neospora caninum* was not performed in 2 (10%) dogs and was negative in the remaining 19 (90%) dogs. In the 2 dogs with lacking infectious disease testing, full necropsy was performed, revealing GME.

Magnetic resonance imaging of the spinal cord was available in all cases, revealing a focal lesion in 15 dogs (71%), a multifocal lesion in 4 dogs (19%) and no lesion was visible on sagittal T2WI or T1WI in 2 dogs (10%). Lesion length was measured in the focal cases only. Median lesion/vertebral body ratio was 4.8 (ranging from 0.6 – 10.9). All visible lesions were ill-defined, intramedullary, hyperintense on T2W images and isointense on T1WI (**Figures 3.1 and 3.2**). Lesions showed parenchymal contrast enhancement in 18 dogs (86%), and contrast enhancement of overlying meninges in 17 dogs (81%) (**Figures 3.3 and 3.4**). In dogs presenting with spinal hyperaesthesia (n=15), there was no significant association with the presence of meningeal contrast enhancement on MRI (P=0.24). In the 2 cases where no lesion was visible on sagittal T2WI and T1WI, no parenchymal contrast enhancement was seen, but 1 of those 2 dogs showed meningeal contrast enhancement. In 2 dogs (10%) intracranial images were present within the field of view of the cervical spinal cord images (T2W transverse and sagittal images), revealing multiple T2W hyperintensities in the forebrain and/or brainstem. Neither of those dogs had clinical or neurological signs of intracranial involvement at time of diagnosis. The first dog, a 56-month-old Jack Russell Terrier, never recovered from general anaesthesia after diagnostic procedures, and full necropsy revealed

GME. The second dog, a 123-month-old Rhodesian Ridgeback, developed seizures 669 days after diagnosis and was euthanized without further investigations.

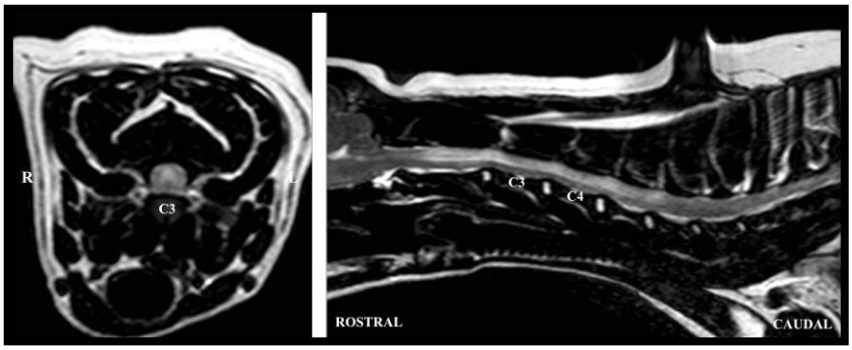

Figure 3.1*: T2W transverse (left image) MR image of the vertebral column and spinal cord at the level of C3, and sagittal (right image) MR image of the cervical and cranial thoracic vertebral column and spinal cord of a 56-month-old Jack Russell terrier. There is presence of a large, ill-defined, intramedullary hyperintensity extending from cranial C2 until cranial C6*.

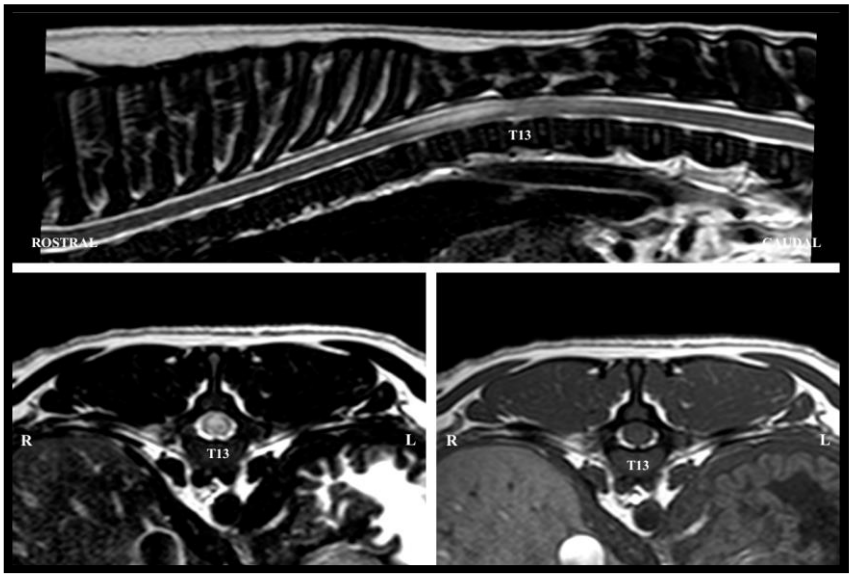

Figure 3.2*: T2W sagittal (top image) and transverse (bottom left image), and pre-contrast T1W transverse (bottom right image) of the vertebral column and associated spinal cord of a 13-month-old French Bulldog. There is presence of a large, ill-defined, intramedullary lesion that is hyperintense on T2W images and isointense on T1W images. the lesion is extending from mid T10 until caudal L1.*

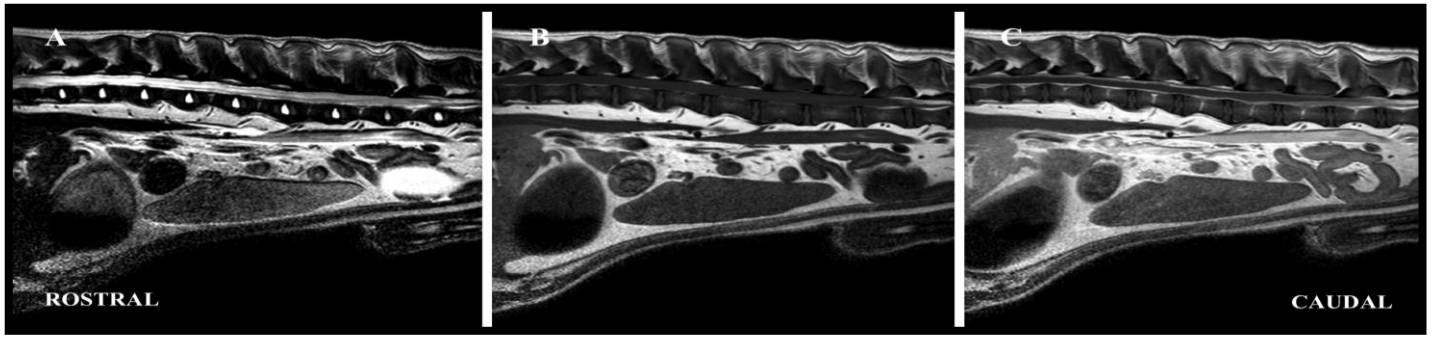

***Figure 3.3**: T2W (A), T1W pre-contrast (B) and T1W post-contrast (C) sagittal MR images of the lumbar spinal cord of a 105-month-old Labrador Retriever. Note the diffuse meningeal enhancement without apparent parenchymal enhancement.*

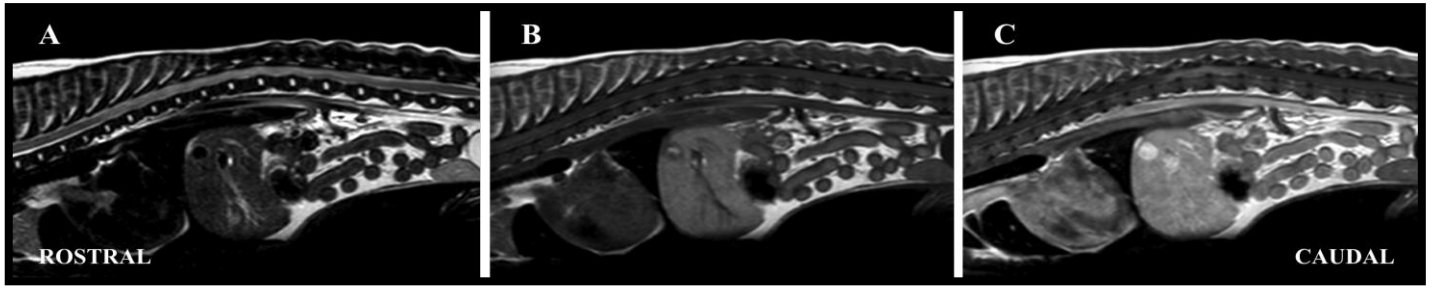

***Figure 3.4**: T2W (A), T1W pre-contrast (B) and T1W post-contrast (C) sagittal MR images of the lumbar spinal cord of a 104-month-old Maltese Terrier. Note the parenchymal enhancement with only minimal meningeal enhancement (mainly rostral to the lesion).*

Treatment and outcome

As required by the inclusion criteria, outcome was available in all dogs. As described above, one dog never recovered from general anaesthesia for MRI of the spinal cord, and this dog was censored for survival analysis. Mean duration of hospitalisation was 5 days (ranging from 1 – 19 days), with 17 dogs (81%) showing improvement in neurological status within those days. One dog (5%) remained neurologically stable (no improvement nor deterioration), and 3 dogs (14%) showed deterioration of their neurological status. All dogs, including the dog that never recovered from anaesthesia, were treated with immunosuppressive doses of glucocorticosteroids immediately after diagnosis. This consisted of IV dexamethasone (dose ranging from 0.3 – 0.5mg/kg/day) in 9 dogs (43%), and oral prednisolone (dose ranging from 2 – 4mg/kg/day) in 12 dogs (57%). Fourteen dogs (67%) received additional treatment with cytosine arabinoside as a CRI of 200mg/m^2 over 8 hours in 1 dog (7%) and as 4 SC injections of 50mg/m^2 every 12 hours for 2 consecutive days in 13 dogs (93%).

Twenty dogs (95%) survived to discharge. Of these dogs, 9 dogs (45%) were still alive at time of data capture. Of these 9 dogs, 8 dogs were neurologically normal according to follow-up information, and 1 dog was still showing ataxia and ambulatory paraparesis. Of the 8 normal dogs, 2 dogs were still receiving ciclosporine 5mg/kg every 24 hours, 1 dog was receiving cytosine arabinoside 50mg/ m^2 every 12 hours for 2 consecutive days every 9 weeks, 1 dog was receiving prednisolone 0.2mg/kg every 24 hours, 1 dog was receiving prednisolone 1mg/kg every 24 hours and cytosine arabinoside 50mg/ m^2 every 12 hours for 2 consecutive days every 4 weeks, and 3 dogs were not receiving any treatment at time of data capture. The dog that was still showing neurological abnormalities was receiving 0.5mg/kg prednisolone every other day and cytosine arabinoside 50mg/ m^2 every 12 hours for 2 consecutive days every 5 weeks. Regarding the 11/20 dogs (55%) that had deceased at time of data capture, 3 dogs died or were euthanized because of disease progression, 6 dogs were euthanized because of acute neurological deterioration after initial neurological improvement, and 2 dogs were euthanized because of unrelated causes (complications after stifle surgery and development of aggression).

Dogs that showed acute neurological deterioration after initial improvement did so within a median of 171 days after diagnosis (ranging from 30-669 days). Of those 6 dogs, 1 dog showed acute deterioration after discontinuation of prednisolone treatment, and 5 dogs were still receiving treatment consisting of prednisolone 1mg/kg every 24 hours, prednisolone 0.5mg/kg every 24 hours, prednisolone 2mg/kg every 24 hours and azathioprine 2mg/kg every 24 hours, or cytosine arabinoside 50mg/m^2 every 12 hours for 2 consecutive days every 7 weeks. Overall, we can conclude that 10/21 dogs (48%) died or were euthanized because of SO-MUA.

No difference was seen in long-term survival between dogs receiving sole prednisolone therapy or combination therapy with cytosine arabinoside (P=0.31). Overall, the MST was 669 days (ranging from 1-2250 days) (Figure 3). No significant difference was seen in relative lesion length on MR imaging between dogs that are alive and dogs that died or were euthanized because of SO-MUA (P=0.91). Post mortem confirmation was available in 3 dogs, revealing GME in 2 dogs and necrotising meningomyelitis in 1 dog. An overview of the treatment schedules, follow-up, ST and necropsy information can be consulted in **table 3.2.**

***Table 3.2**: Treatment and outcome details of 21 dogs diagnosed with SO-MUA. SC = subcutaneous, CRI = constant rate infusion, PM = post mortem.*

Case	Breed	Initial treatment	Cytosine arabinoside dose (mg/m^2), SC or CRI	Initial response to treatment	Long-term follow-up and treatment	Death because of SO-MUA	ST (days)	PM
1	Akita	Prednisolone 2mg/kg /day	50mg/m^2 SC	Improvement	Euthanasia because of acute deterioration after discontinuation of prednisolone treatment	Yes	380	NP
2	Rottweiler	Prednisolone 2mg/kg /day	50mg/m^2 SC	Deterioration	Euthanasia because of disease progression	Yes	20	NP
3	Bull Mastiff	Prednisolone 2mg/kg /day	No cytosine arabinoside	Deterioration	Euthanasia because of disease progression	Yes	6	NP
4	Labrador Retriever	Dexamethasone 0.3mg/kg/day	50mg/m^2 SC	Improvement	Euthanasia because of acute deterioration, was still receiving 1mg/kg of prednisolone every day	Yes	30	NP
5	Jack Russell Terrier	Prednisolone 2mg/kg /day	No cytosine arabinoside	Improvement	Normal dog, still receiving 0.2mg/kg/day prednisolone	No	237	NA

Case	Breed	Initial treatment	Cytosine arabinoside dose (mg/m^2), SC or CRI	Initial response to treatment	Long-term follow-up and treatment	Death because of SO-MUA	ST (days)	PM
6	Lhasa Apso	Prednisolone 4mg/kg /day	50mg/m^2 SC	Improvement	Euthanasia because of acute deterioration, was still receiving 0.5mg/kg prednisolone per day	Yes	171	GME
7	Shih Tzu	Prednisolone 2mg/kg /day	50mg/m^2 SC	Improvement	Normal dog, receiving ciclosporine 5mg/kg/day	No	2250	NA
8	Giant Schnauzer	Prednisolone 2mg/kg /day	50mg/m^2 SC	Improvement	Euthanasia because of aggression, was only receiving cytosine arabinoside every 5 weeks	No	752	NP
9	Yorkshire Terrier	Prednisolone 2mg/kg /day	No cytosine arabinoside	Improvement	Euthanasia because of acute deterioration, was still receiving 1mg/kg of prednisolone per day	Yes	202	NME

Case	Breed	Initial treatment	Cytosine arabinoside dose (mg/m^2), SC or CRI	Initial response to treatment	Long-term follow-up and treatment	Death because of SO-MUA	ST (days)	PM
10	English Springer Spaniel	Prednisolone 2mg/kg /day	No cytosine arabinoside	Improvement	Euthanasia because of post-operative infection after stifle surgery, dog normal and on no medication	No	304	NP
11	Rhodesian Ridgeback	Dexamethasone 0.3mg/kg/day	50mg/m^2 SC	Improvement	Euthanasia because development of seizures, was still receiving cytosine arabinoside 50mg/m^2 SC every 7 weeks	Yes	669	NP
12	Bearded Collie	Prednisolone 2mg/kg /day	50mg/m^2 SC	Improvement	Normal dog, receiving no current treatment	No	1100	NA
13	Boxer	Prednisolone 2mg/kg /day	50mg/m^2 SC	Improvement	Normal dog, receiving cytosine arabinoside 50mg/m^2 SC every 9 weeks	No	1460	NA
14	Lhasa Apso	Prednisolone 2mg/kg /day	50mg/m^2 SC	Stable	Euthanasia because of disease progression	Yes	33	NP

Case	Breed	Initial treatment	Cytosine arabinoside dose (mg/m^2), SC or CRI	Initial response to treatment	Long-term follow-up and treatment	Death because of SO-MUA	ST (days)	PM
15	Chihuahua	Dexamethasone 0.3mg/kg/day	No cytosine arabinoside	Improvement	Normal dog, receiving no current treatment	No	635	NA
16	Cross Breed	Dexamethasone 0.3mg/kg/day	200mg/m^2 CRI	Improvement	Euthanasia because of acute deterioration, was still receiving 2mg/kg of prednisolone every day, combined with 2mg/kg azathioprine	Yes	93	NP
17	French Bulldog	Prednisolone 2mg/kg/day	No cytosine arabinoside	Improvement	Normal dog, receiving no current treatment	No	791	NA
18	Maltese Terrier	Dexamethasone 0.3mg/kg/day	50mg/m^2 SC	Improvement	Normal dog, still receiving 1mg/kg of prednisolone per day, and cytosine arabinoside 50mg/m^2 SC every 4 weeks	No	577	NA

Case	Breed	Initial treatment	Cytosine arabinoside dose (mg/m^2), SC or CRI	Initial response to treatment	Long-term follow-up and treatment	Death because of SO-MUA	ST (days)	PM
19	Jack Russell Terrier	Dexamethasone 0.5mg/kg/day	No cytosine arabinoside	Dog never recovered from general anaesthesia for MRI	Dog never recovered from general anaesthesia	Yes	0	GME
20	French Bulldog	Dexamethasone 0.3mg/kg/day	50mg/m^2 SC	Improvement	Ataxia and ambulatory paraparesis, still receiving 0.5mg/kg of prednisolone every other day and cytosine arabinoside 50mg/m2 every 5 weeks	No	90	NP
21	West Highland White Terrier	Dexamethasone 0.3mg/kg/day	50 mg/m^2 SC	Improvement	Normal dog, receiving ciclosporine 5mg/kg/day	No	210	NA

Discussion

This study evaluated the clinical presentation, diagnostic findings and long-term survival in 21 dogs diagnosed with presumptive SO-MUA. Dogs had a median age of 5 years at time of diagnosis. A lesion affecting the T3-L3 spinal cord segments resulting in ambulatory paraparesis was considered the most common clinical presentation. Although the overall MST was 669 days, 48% of dogs diagnosed with SO-MUA died or were euthanized because of this disease, indicating a guarded long-term prognosis.

To be included in the study, dogs were not allowed to have clinical signs or neurological examination abnormalities suggestive of intracranial involvement. Interestingly, additional MR images of the brain were included in the field of view of the cervical MRI in 2 dogs, showing additional lesions in both cases. One of those dogs, a 123-month-old Rhodesian Ridgeback, developed seizures 669 days after diagnosis despite on-going cytosine arabinoside treatment, and was therefore euthanized. No necropsy was performed, but because intracranial lesions were already present at time of diagnosis, development of MUA was assumed. The other dog, a 56-month-old Jack Russell Terrier, never recovered from general anaesthesia for MR imaging. Necropsy was performed, revealing the presence of GME. Because intracranial MR images were only available in 2 dogs, it is currently unclear (1) if these brain abnormalities represent a multifocal nature of the disease or cranial extension of the cervical inflammatory lesions, and (2) if inflammatory brain lesions are currently under diagnosed in dogs with SO-MUA and if SO-MUA could therefore be considered a more generalised inflammatory disease process, a meningoencephalomyelitis.

Pain on direct spinal palpation was present in 71% of dogs. Spinal pain reflects the involvement of the meninges, and/or vertebrae (vertebral periosteum), and/or nerve roots or spinal nerves (Da Costa, 2012). In the present study, the lesions showed meningeal contrast enhancement in 18/21 dogs, but there was no significant association between spinal hyperaesthesia and the presence of meningeal enhancement on MRI.

MRI of the spinal cord revealed no lesion on sagittal T2WI and T1WI in 10% of dogs (n=2), which appears similar to the 7% described for the brain in dogs with MUA (Granger et al., 2010). In the retrospective study of Griffin et al. (2008), only 1 dog with meningomyelitis had MRI performed, revealing no abnormalities. Based on these findings, presence of SO-MUA cannot be ruled out based on unremarkable MRI findings. The first dog was a 42-month-old Bull Mastiff with a one-month history of slowly progressive T3-L3 spinal cord lesion. After diagnostic procedures, the dog was treated with oral prednisolone but continued to deteriorate and was euthanized after 6 days. No necropsy was performed. The second dog was a 136-month-old Bearded Collie with a one-week history of a progressive multifocal spinal cord neuroanatomical localisation (T3-S3 spinal cord lesion). The dog showed improvement on treatment with prednisolone and cytosine arabinoside (see **table 3.2**) after diagnostic investigations, and was still alive without current treatment 1100 days after diagnosis. Both dogs had inflammatory CSF analysis (increased TNCC and TP concentration). For both dogs, the presence of vascular, degenerative and neoplastic spinal cord lesions cannot be excluded. As both dogs had a progressive disease course, a vascular (ischaemic) lesion seemed less likely. A neoplastic lesion cannot be excluded, although this seems rather unlikely in the Bull Mastiff considering his young age. The second dog had a lymphocytic pleocytosis on CFS analysis, but no signs of lymphoma were seen on microscopical examination, however no specific test to look for clonality was performed.

If a lesion was visible on MRI, all lesions were extensive, ill-defined, intramedullary, hyperintense on T2WI and isointense on T1WI. Other spinal conditions, including acute non-compressive nucleus pulposus extrusion (ANNPE) and ischaemic myelopathy (IM), are also associated with intraparenchymal hyperintensities on MRI. These conditions are however associated with other clinical and MRI characteristics, which could potentially aid in differentiating between these conditions (Cardy et al., 2015; Fenn et al., 2016). Looking into a recent study (Cardy et al., 2015), the clinical presentation of dogs with spinal cord dysfunction, IM (most commonly fibrocartilagenous embolic myelopathy (FCEM)) and ANNPE are typically characterised by a peracute onset of non-progressive clinical signs and affected dogs do not

commonly demonstrate overt spinal hyperaesthesia at time of admission. This is in contrast with the clinical presentation of dogs with SO-MUA, which was characterised by an acute onset of progressive and mainly symmetrical neurological deficits, with pain on spinal palpation or manipulation in 86% of dogs (Cardy et al., 2015), which is comparable with the 71% of dogs presenting with spinal hyperaesthesia in the presented study. Although CSF analysis in dogs with IM is most often within normal limits, affected dogs can demonstrate an increased TP concentration and mild neutrophilic or mixed cell pleocytosis with a median TNCC of 12 WBC/mm^3 (De Risio et al., 2007). A marked pleocytosis with a median TNCC of 209 WBC/mm^3 was seen in the presented study, although results should be interpreted with caution as presence of a CSF pleocytosis was considered one of the inclusion criteria. To conclude, the presentation of a dog with an acute or chronic onset of a progressive and painful T3-L3 myelopathy in combination with an extensive, ill defined, intramedullary lesion with presence of parenchymal and/or meningeal contrast enhancement on MRI, and presence of a marked pleocytosis on CSF analysis, can be presumptively diagnosed with SO-MUA. The importance of differentiating between these conditions is highlighted by the differences in treatment and prognosis between dogs with presumptive SO-MUA and dogs with ANNPE or IM.

A previous study demonstrated that short tau inversion recovery (STIR) hyperintensities in the cervical epaxial musculature of dogs with meningoencephalomyelitis had a sensitivity of 78% and a specificity of 92% in predicting inflammatory CSF results (Eminaga et al., 2013). In the presented study, STIR images were unfortunately only available in 3/21 cases. Adding this sequence to the protocol in dogs with presence of a focal or multifocal, ill-defined T2W intramedullary hyperintensity might be considered in the future.

Several studies have evaluated survival times of dogs diagnosed with MUA (Granger et al., 2010, Coates and Jeffery, 2014). Overall, dogs with MUA appear to have a guarded prognosis. A large systematic review of dogs with MUA revealed an overall reported MST of 240-590 days in 96 dogs treated with corticosteroids plus any other immunosuppressive protocol, compared to a MST of 28-357 days for 43 dogs receiving corticosteroids alone (Granger et al.,

2010). In the presented study, dogs with presumptive SO-MUA had a MST of 669 days (2 years), but ultimately, 48% of dogs died or were euthanized because of SO-MUA, indicating a more guarded long-term prognosis.

Limitations of this study are the relative small sample size and retrospective character, which limited standardisation of patient assessment and treatment. Although dogs were all treated with glucocorticosteroids, it cannot be excluded that specific differences in treatment have influenced our results. Despite including cases over a relative large period and from a busy referral hospital, only 21 dogs could be included. This could indicate that SO-MUA should be considered a rare disorder.

Conclusions

SO-MUA can be diagnosed in every dog breed of every age that is presented with signs of a mainly acute or chronic, possibly painful, myelopathy. Although clinical signs can vary, affected animals most typically present with ambulatory paraparesis and ataxia, localizing to T3-L3 spinal cord segments. MRI typically reveals an extensive, ill-defined and intramedullary lesion that appears hyperintense on T2WI and isointense on T1WI. Most lesions showed parenchymal contrast enhancement and/or enhancement of the overlying meninges on post-contrast T1WI which can possibly differentiate dogs with SO-MUA from other more common spinal diseases. In 10% of cases, no lesion was visible on sagittal T2WI and T1WI. Almost 50% of dogs died or were euthanized because of SO-MUA, with a MST of 669 days for all dogs. Future studies should be performed looking into intracranial imaging in dogs diagnosed with presumptive SO-MUA and its prognostic value, extensive infectious disease testing in all cases and outcome using a standard treatment protocol to give more information about this condition.

Part II

Treatment Options

Chapter 3

SOLE PREDNISOLONE THERAPY IN CANINE MENINGOENCEPHALOMYELITIS OF UNKNOWN AETIOLOGY

Ine Cornelis[a], Luc Van Ham[a], Steven De Decker[b], Kaatje Kromhout[c], Klara Goethals[d], Ingrid Gielen[c], Sofie Bhatti[a]

[a]*Small Animal Department, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium.*
[b]*Clinical Science and Services, The Royal Veterinary College, University of London, Hatfield, United Kingdom.*
[c]*Department of Veterinary Medical Imaging and Small Animal Orthopaedics, Ghent University, Merelbeke, Belgium*
[d]*Department of Comparative Physiology and Biometrics, Ghent University, Merelbeke, Belgium*

Adapted from: Cornelis I, Van Ham L, De Decker S, Kromhout K, Goethals, K, Gielen I, Bhatti S (2017). Sole prednisolone therapy in canine meningoencephalitis of unknown aetiology. *Vlaams Diergeneeskundig Tijdschrift* 86, 24-28.

Abstract

MUA is a frequently diagnosed and often fatal disease in veterinary neurology. The aim of this retrospective study was to assess the efficacy of 3 different sole prednisolone treatment schedules in dogs diagnosed with MUA. Dogs were diagnosed clinically with MUA based on previously described inclusion criteria, and treated with a 3, 8 or 18-week tapering prednisolone schedule. Thirty-eight dogs were included in the study, and 17, 15 and 6 dogs received the 3, 8 and 18-week tapering schedule, respectively. Overall, 37% of dogs died or were euthanized because of MUA, and a significant difference in survival time was seen between the three treatment schedules. Surprisingly, the highest number of dogs that died because of MUA was seen in the 8-week treatment schedule (56%), followed by the 3-week (26%) and 18-week (0%) treatment schedule. Based on the results of this study, no definitive conclusions can be drawn regarding the ideal prednisolone dosing protocol for dogs diagnosed with MUA, but a more aggressive and immunosuppressive treatment protocol might lead to a better outcome.

Introduction

MUA is a group of non-infectious central nervous system diseases, with a likely multifactorial pathogenesis (Coates et al., 2014). Making a definitive diagnosis requires histopathological examination of central nervous tissue, but a presumptive ante-mortem clinical diagnosis can be achieved based on a combination of neurological examination results, MRI findings and CSF abnormalities (Coates et al., 2014). The exact aetiology and pathophysiology of MUA are currently unknown, but the cornerstone of medical treatment is considered to be immunosuppressive drugs. Several treatment protocols with different associated long-term survival times have been reported, whereby treatment with glucocorticosteroids only is generally associated with shorter survival times (Munana and Luttgen, 1998; Jung et al., 2007; Granger et al., 2010; Flegel et al., 2011; Mercier and Barnes Heller, 2015) compared to combination therapy with other immunosuppressive therapies, including cytosine arabinoside, ciclosporine, leflunomide, lomustine, azathioprine, procarbazine, mycophenolate mofetil, vincristine and cyclophosphamide or radiation therapy (Sisson et al. 1989; Gregory et al., 1998; Munana and Luttgen, 1998; Adamo and O'Brien, 2004; Gnirs, 2006; Zarfoss et al., 2006; Adamo et al., 2007; Coates et al., 2007; de Stefani et al., 2007; Feliu-Pascual et al., 2007; Uriarte et al., 2007; Jung et al., 2007; Menaut et al., 2008; Pakozdy et al., 2009; Smith et al., 2009; Granger et al., 2010; Kang et al., 2009; Wong et al., 2010; Flegel et al., 2011; Jung et al., 2012; Jung et al., 2013; Lowrie et al., 2013; Beckmann et al., 2015; Mercier and Barnes Heller, 2015; Barnoon et al., 2015; Lowrie et al., 2016). However, in a clinical setting, adding more expensive immunosuppressive therapies to the glucocorticoid protocol might be financially impossible. A recently published prospective trial in dogs with MUA reported an overall median survival time of 602 days for dogs receiving immunosuppressive doses of glucocorticosteroids starting at a dose of 1mg/kg twice daily, supporting the use of monotherapy with glucocorticosteroids in the treatment of MUA (Mercier and Barnes Heller, 2015). Therefore, the aims of this study were to retrospectively evaluate the efficacy of 3 different prednisolone treatment protocols that were historically used (3, 8 and 18 week tapering schedule) in dogs diagnosed with MUA. We hypothesized that a longer survival time would be achieved with a longer and more immunosuppressive treatment protocol.

Materials and methods

The electronic medical database of Ghent University, Small Animal Department, was searched between March 2006 and September 2014, and owner contact was performed in October 2014. Adapted inclusion criteria were used (Granger et al., 2010), considering dogs suitable for inclusion if following data were available: (1) signalment, (2) localisation by neurological examination, (3) inflammatory CSF analysis, (4) intracranial MR and/or CT imaging results, (5) negative infectious disease testing, and (6) long-term follow-up through research of medical records or through telephone contact with the owner or referring veterinarian. Neurological status was recorded at time of admission, and further on a daily basis. Results were recorded daily in a computer program if the dog was hospitalized. Outcome was defined as successful if dogs were not showing the previously reported neurological signs or if improvement was seen according to the owner or referring veterinarian. Unsuccessful outcome was defined as death or euthanasia because of disease progression or if no change in neurological signs was seen. Relapse was defined as a sudden deterioration in neurological status after an initial improvement after diagnosis and initiation of treatment. All dogs were only receiving glucocorticosteroids as immunomodulating therapy, and a tapering prednisolone treatment schedule consisting of 3, 8 or 18 weeks was used (**table 4.1**).

***Table 4.1**: Three different oral prednisolone treatment schedules*

3 weeks	8 weeks	18 weeks
1 week 1mg/kg q24h	2 weeks 1mg/kg q12h	3 weeks 1.5mg/kg q12h
1 week 0.5mg/kg q24h	2 weeks 0.5mg/kg q12h	6 weeks 1mg/kg q12h
1 week 0.25mg/kg q24h	2 weeks 0.5mg/kg q24h	3 weeks 0.5mg/kg q12h
	2 weeks 0.25mg/kg q24h	3 weeks 0.5mg/kg q24h
		3 weeks 0.25mg/kg q24h

Dogs treated with the 3-week schedule were diagnosed between March 2006 and March 2010, dogs receiving the 8-week schedule between January 2009 and August 2012, and dogs receiving the 18-week schedule between January 2010 and September 2014. The 18-week treatment schedule started with an immunosuppressive dose of prednisolone, being 3mg/kg/day, compared to the 8-week schedule that started at an immunosuppressive dose of 2mg/kg/day. The 3-week tapering schedule started with an anti-inflammatory dose of 1mg/kg/day. It was recorded whether dogs survived their initial treatment protocol and whether a relapse in neurological signs (sudden deterioration after initial improvement) was seen during treatment and the associated changes made to the prednisolone schedule. ST was defined as time from diagnosis to death or euthanasia. A semiparametric Cox model (hazard analysis) was fitted to the data to detect differences in survival time between the three treatment groups. All statistical tests were performed using S-Plus. For all analyses, a value of $P<0.05$ was considered significant.

Results

Thirty-eight dogs met the inclusion criteria. Breeds represented included Maltese terrier (n=10), Yorkshire terrier (n=3), Golden Retriever (n=3), Chihuahua (n=3), French bulldog (n=3), Pug (n=2), Labrador retriever (n=2), West Highland White terrier (n=2), Shih Tzu (n=2), Boston terrier (n=2), American Staffordshire terrier (n=2) and 4 other individual breeds. Most common presenting neurologic signs were abnormal behaviour (n=19), altered mentation (n=18) and central vestibular signs (n=19). Brain imaging (CT and/or MRI) was available in all cases. Thirteen dogs (34%) only underwent CT imaging (**Figure 4.1**), 24 dogs (63%) only underwent MR imaging and 1 dog (3%) underwent both CT and MR imaging. No lesion was visible in 7 dogs (18%), based on CT (n=4) or MR (n=3) imaging. As required by the inclusion criteria, TNCC of the CSF was above reference limits (> 5 WBC/mm^3 after cisternal collection) in all cases with counts ranging from 9-1189 WBC/mm^3 (median: 55 WBC/mm^3). An overview of the most important diagnostic findings can be consulted in **table 4.2**.

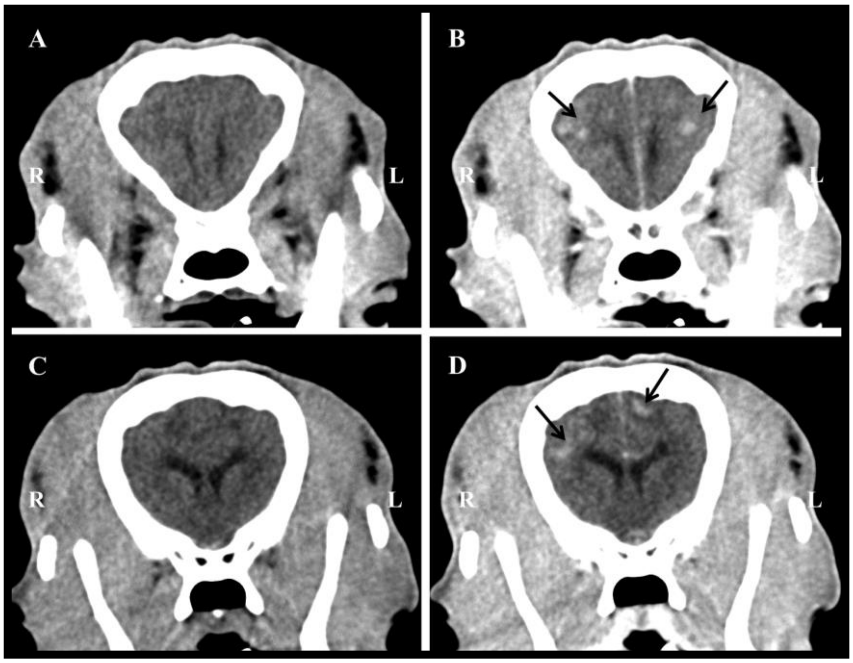

Figure 4.1. *CT images at the level of the frontal lobe (A and B) and caudate nucleus (C and D) of an 8-month-old female entire French Bulldog. Images B and D are acquired after administration of IV gadolinium contrast. No lesions were visible on the pre-contrast CT images (A and C), but multiple hyperdense, rounded, intraaxial lesions were visible within the frontal (B) and parieto-temparal (D) cerebral cortex (arrows) of the dog.*

***Table 4.2**: Overview of the most important diagnostic findings in the included cases. Abbreviations: TNCC = total nucleated cell count, WBC = white blood cells, CT = computed tomography, MRI = magnetic resonance imaging, ST = survival time*

Case	Breed	TNCC (WBC/ mm^3)	Imaging modality	Description imaging findings	Prednisolone schedule (weeks)	ST (days)
1	American Staffordshire Terrier	165	CT	No lesion visible	3	2190
2	American Staffordshire Terrier	52.25	MRI	No lesion visible	3	1167
3	Boston Terrier	24	MRI	Diffuse	8	1460
4	Boston Terrier	15	MRI	Diffuse	8	30
5	Chihuahua	30	MRI	Focal forebrain	8	8
6	Chihuahua	9	MRI	Diffuse	18	730
7	Chihuahua	16.5	MRI	Focal cerebellum	18	70

Case	Breed	TNCC (WBC/ mm^3)	Imaging modality	Description imaging findings	Prednisolone schedule (weeks)	ST (days)
8	German Shepherd	300	MRI	Diffuse	8	10
9	Miniature Schnauzer	30.25	MRI	Focal forebrain / thalamus	8	778
10	French bulldog	22	CT	Diffuse	3	61
11	French bulldog	66	MRI	Focal brainstem	8	1275
12	French bulldog	25	MRI	Multifocal brainstem and forebrain	18	365
13	Golden Retriever	118	CT	No lesion visible	3	370
14	Golden Retriever	20	MRI	Focal brainstem	3	1095
15	Golden Retriever	42.6	MRI	Diffuse	18	120

Case	Breed	TNCC (WBC/ mm^3)	Imaging modality	Description imaging findings	Prednisolone schedule (weeks)	ST (days)
16	Labrador Retriever	209	MRI	Diffuse	3	1095
17	Labrador Retriever	1189	MRI	Diffuse	8	150
18	Maltese terrier	74	CT	No lesion visible	3	2190
19	Maltese terrier	66	CT	Diffuse	3	2310
20	Maltese terrier	12.4	CT	Diffuse	3	2035
21	Maltese terrier	800	CT	Diffuse	3	2
22	Maltese terrier	21	MRI	Diffuse	8	13
23	Maltese terrier	64	MRI	Diffuse	8	84
24	Maltese terrier	96.25	MRI	Diffuse	8	180

Case	Breed	TNCC (WBC/ mm^3)	Imaging modality	Description imaging findings	Prednisolone schedule (weeks)	ST (days)
25	Maltese terrier	120	MRI	Diffuse	8	1275
26	Maltese terrier	15	MRI	No lesion visible	18	730
27	Maltese terrier	50	MRI	Multifocal brainstem and forebrain	18	365
28	Pug	217	CT	Diffuse	3	185
29	Pug	52.25	CT	Diffuse	3	1095
30	Shih Tzu	82.5	MRI and CT	Diffuse	3	1921
31	Shih Tzu	55	MRI	No lesion visible	8	72
32	Tervueren Shepherd	27	MRI	Diffuse	8	210

Case	Breed	TNCC (WBC/ mm^3)	Imaging modality	Description imaging findings	Prednisolone schedule (weeks)	ST (days)
33	Weimaraner	110	MRI	Diffuse	8	72
34	West Highland White terrier	33	CT	No lesion visible	3	730
35	West Highland White terrier	500	MRI	Diffuse	8	545
36	Yorkshire terrier	33	CT	Diffuse	3	2490
37	Yorkshire terrier	143	CT	Diffuse	3	2490
38	Yorkshire terrier	500	CT	Diffuse	3	6205

Seventeen (45%), 15 (39%), and 6 (16%) dogs received the 3, 8, and 18-weeks prednisolone treatment schedule, respectively. In 8 dogs (21%) this therapy was combined with phenobarbital for treatment of seizures. There was a significant difference in ST between the three treatment groups (P=0.028) (**Figure 4.2**).

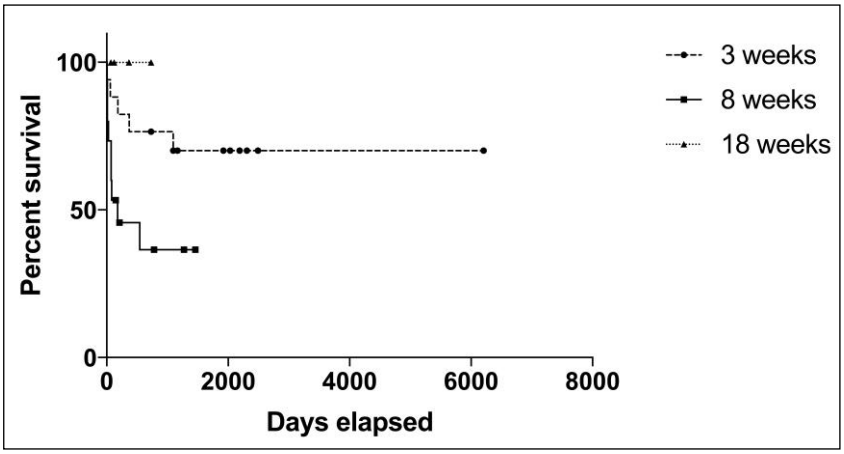

***Figure 4.2**: Kaplan-Meier survival curve comparing survival times in the three treatment groups. Dogs that were still alive at time of data capture, or died because of unrelated causes, were censored for survival analysis.*

Overall, 24 dogs (63%) had a successful outcome, 22 of those dogs were alive at time of data capture. The remaining 2 dogs were euthanized because of other reasons 730 and 1167 days after their diagnosis, respectively, and both dogs were not receiving any immunomodulating medication at time of euthanasia. Fourteen dogs (37%) had an unsuccessful outcome, and died or were euthanized because of MUA. The MST could not be calculated for the group of dogs receiving the 3-week and 18-week tapering prednisolone schedule, as more than 50% of those dogs was alive at time of data capture. The MST for the dogs treated with the 8-week tapering schedule, was 180 days. Five dogs died or were euthanized (1 in the 3-week group en 4 in the 8-week group) during their treatment schedule and no changes were made to their schedule. Ten dogs (26%) showed a relapse in neurological signs, 4 of those dogs did so after terminating their 3-week tapering schedule, 3 dogs after

terminating their 8-week treatment schedule, 2 dogs during their 8-week schedule (after 3 and 6 weeks), and 1 dog after terminating the 18-week schedule. If the relapse was seen after termination of the treatment schedule, the same schedule was initiated again (n=7), and the dose was increased again to the starting dose if a relapse was seen before termination of the tapering schedule (n=3). There was no significant difference in relapse rates between the three treatment schedules (P=0.886). An overview of the results can be found in **table 4.3**.

***Table 4.3**: Summary of survival, relapse and MST in dogs within the treatment groups. NR = not related (dog died for reason unrelated to MUA)*

	3 weeks	**8 weeks**	**18 weeks**
Number of dogs	17	15	6
Dead	5 (2 NR)	9	0
Alive	10	6	6
Deceased cases			
During protocol	1	4	0
After protocol	4	5	0
Relapsed cases	4	5	1
During protocol	0	2	1
After protocol	4	3	0
MST	-	180 days	-

Discussion

Thirty-eight dogs diagnosed with MUA received oral prednisolone therapy in 3 different tapering schedules. Overall, 37% of dogs were euthanized because of MUA, and survival curves for the three treatment schedules were significantly different. Surprisingly, the highest mortality rate was seen in the 8-week (immunosuppressive) treatment group (56%), followed by the 3-week (anti-inflammatory) (28%) and the 18-week (immunosuppressive) treatment (0%) schedule. Possible explanations might be that (1) all dogs at the institution historically received the 3-weeks treatment protocol when a suspicion of non-infectious encephalitis was made. However, as new literature became available on a possibly immune-mediated origin, dogs diagnosed with MUA admitted with severe neurological signs may have received a longer, more immunosuppressive (8-weeks) treatment schedule and (2) the 18-week tapering schedule was only introduced in the last 4 years of inclusion, so the recently included cases might still decease in the following weeks to months, now falsely (positively) influencing the results.

In literature, the MST in dogs diagnosed with MUA and receiving sole prednisolone therapy ranges from 28-357 days (Granger et al., 2010), 91-329 days (Flegel et al., 2011) and 602 days (Mercier and Barnes Heller, 2015). As more than 50% of the dogs was alive or censored for outcome calculations at time of data capture, no overall MST could be calculated in the presented study. However, the MST was 180 days in the 8-week treatment group, which appears to be the group with the highest percentage of deceased and relapsed dogs. Further prospective studies should be performed, including more dogs receiving the more immunosuppressive treatment schedules, although one can have ethical problems with comparing an immunosuppressive and anti-inflammatory treatment protocol for a presumed immune-mediated disease.

Pitfalls of the current study are the lack of histopathological confirmation in all cases, the low number of cases and the relatively high percentage of dogs that was diagnosed using CT imaging (34%). However 18%

of dogs had no visible lesion on CT or MR imaging which is comparable to the 7% for MRI and 14% for CT described previously (Granger et al., 2010). Prednisolone therapy is associated with common side effects, and to overcome these, other immunosuppressive drugs can be added to the protocol. Looking at the side effects of the prednisolone treatment was beyond the scope of this article, as not all side effects were systematically recorded and these are difficult to trace back through telephone contact with the owner or referring veterinarian.

To conclude, overall prognosis for dogs diagnosed with MUA and treated with sole prednisolone therapy is guarded. Almost 40% of dogs will succumb due to the disease. A long and immunosuppressive treatment schedule is advised, but larger studies are needed to support this.

Chapter 4

SOLE PREDNISOLONE THERAPY VERSUS COMBINATION THERAPY WITH CICLOSPORINE IN DOGS WITH MENINGOENCEPHALOMYELITIS OF UNKNOWN AETIOLOGY

Ine Cornelis[a], Ingrid Gielen[b], Luc Van Ham[a], Valentine Martlé[a], Kenny Bossens[a], Bart Broeckx[c], Emilie Royaux[a], Kimberley Stee[a], Sofie Bhatti[a]

[a]*Small Animal Department, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium.*
[b]*Department of Veterinary Medical Imaging and Small Animal Orthopaedics, Ghent University, Merelbeke, Belgium.*
[c]*Department of Nutrition, Genetics and Ethology, Ghent University, Merelbeke, Belgium.*

Adapted from: Cornelis I, Gielen I, Van Ham L, Martlé V, Bossens K, Royaux E, Stee K, Bhatti S. Sole prednisolone therapy versus combination therapy with ciclosporine in dogs with meningoencephalomyelitis of unknown aetiology: investigation of prognostic factors and outcome. In preparation for submission.

Abstract

Although ciclosporine has previously been investigated as sole or add-on treatment for dogs diagnosed with MUA, no specific treatment protocols have been defined. The aims of this study were 1) to compare sole prednisolone therapy with combination therapy with ciclosporine, 2) to describe two clinically useful treatment protocols, and 3) to evaluate previously investigated prognostic factors.

A prospective, randomized clinical study including client-owned dogs was performed. Dogs were clinically diagnosed with MUA using previously described inclusion criteria. Dogs were re-admitted 2, 4 and 6 months after diagnosis for a complete neurological examination and analysis of cerebrospinal fluid. Four months after diagnosis, MR imaging was repeated. Twelve dogs met the inclusion criteria. Seven dogs were treated with a sole prednisolone treatment schedule, and 5 dogs with combination therapy of prednisolone and ciclosporine. A previously described 18-week tapering immunosuppressive prednisolone treatment protocol was compared with combination of the same protocol with ciclosporine. Treatment resulted in a MST of 87 and 567.5 days for dogs treated with sole prednisolone and combination therapy, respectively, but no difference in survival curves was found between both treatment groups. Previously established prognostic factors including duration of clinical signs prior to diagnosis, presentation within one week of onset of clinical signs, presence of seizures or abnormal mentation at time of diagnosis, presence of focal versus multifocal signs and presence of focal forebrain versus focal brainstem signs at time of diagnosis, results of CSF analysis, and MRI findings including mass effect, loss of cerebral sulci and foramen magnum herniation, were not associated with outcome in the presented study.

Overall, 67% of dogs died because of MUA. Two clinically useful treatment protocols were described, but no significant difference in MST could be demonstrated comparing both protocols. Additionally, previously established prognostic factors could not be confirmed.

Introduction

Meningoencephalomyelitis of unknown aetiology (MUA) encompasses a group of idiopathic, non-infectious CNS diseases that lack histopathological confirmation (Granger et al., 2010; Talarico and Schatzberg, 2010; Coates and Jeffery, 2014). A clinical diagnosis of MUA can be achieved based on a combination of signalment, neurological examination results, MRI findings and CSF analysis (Munana and Luttgen, 1998; Adamo et al., 2007; Granger et al., 2010; Talarico and Schatzberg, 2010; Coates and Jeffery, 2014). The condition is considered fatal without initiation of appropriate treatment (Munana and Luttgen, 1998; Granger et al., 2010). Conditions comprising MUA, including GME, NLE and NME, are considered immune-mediated diseases, and the cornerstone of medical treatment is immunosuppressive therapy (Sorjonen, 1990; Vandevelde et al., 1981; Kipar et al; 1998; Wong et al., 2010). Several treatment protocols with different associated long-term survival times have been reported, whereby treatment with glucocorticosteroids only is generally associated with shorter survival times (Munana and Luttgen, 1998; Jung et al., 2007; Pakozdy et al., 2009; Granger et al., 2010; Flegel et al., 2011; Mercier and Barnes Heller, 2015) compared to combination therapy with other immunosuppressive therapies.

Ciclosporine therapy has been described as sole treatment for MUA (Adamo and O'Brien, 2004; Adamo et al., 2007), or as combination therapy with prednisolone (Adamo and O'Brien, 2004; Gnirs, 2006; Adamo et al., 2007; Jung et al., 2007; Pakozdy et al., 2009; Kang et al., 2009; Jung et al., 2012; Jung et al., 2013), ketoconazole (Adamo et al, 2007), or cytosine arabinoside and prednisolone (Behr et al., 2009). Only 2 prospective studies are currently available (Jung et al., 2007; Pakozdy et al., 2009) evaluating sole prednisolone therapy and combination therapy with ciclosporine. Jung et al. (2007) included 3 dogs on sole prednisolone therapy and 4 dogs receiving combination therapy, and found a mean survival of 58.3 and 305.7 days for sole prednisolone and combination therapy, respectively. Pakozdy et al. (2009) included 7 dogs in each treatment group, resulting in significantly different MSTs, 28 days for sole

prednisolone therapy and 620 days for combination therapy. No clinically applicable treatment schedule has been described in both studies.

Because MUA generally has a guarded prognosis, multiple studies attempted to identify prognostic factors, often resulting in conflicting results. Currently, the following established prognostic factors for dogs with MUA are available in the literature: 1) younger age at time of diagnosis was significantly associated with improved survival (Oliphant et al., 2016), 2) presence of seizures or altered mentation were significantly associated with shorter survival (Bateman and Parent, 1999; Coates et al., 2007; Granger et al., 2010); 3) presentation within 7 days of onset of clinical signs was significantly associated with longer survival (Barnoon et al., 2015); 4) abnormal serial CSF analysis was significantly associated with relapse and poor outcome (Lowrie et al., 2013); and 5) mass effect, loss of cerebral sulci and foramen magnum herniation on MRI were all significantly associated with death within 3 months (Lowrie et al., 2013).

The aims of this study were 1) to compare sole prednisolone therapy with combination therapy with ciclosporine, 2) to describe two clinically useful treatment protocols, and 3) to investigate possible prognostic factors including clinical factors and factors identified on serial MRI and CSF analysis. We hypothesized that combination therapy with ciclosporine, and resolution/improvement of MRI lesions and/or CSF abnormalities would lead to an improved survival and a decreased risk for relapse.

Materials and methods

Case selection

A prospective recruitment of dogs diagnosed clinically with MUA was performed at Ghent University between January 2014 and September 2015. Dogs were included based on the criteria used by Granger et al. (2010), if they had (1) a complete neurological examination performed leading to a focal or multifocal intracranial neuroanatomical localisation, (2) MR imaging of the brain

demonstrating single, multiple or diffuse intra-axial hyperintense lesions on FLAIR and T2WI, (3) cisternal CSF examination results, and if (4) infectious disease testing returned negative. The study was approved by the Ethical Committee of Ghent University (EC 2013/103) and all owners signed an informed consent form before inclusion. Parameters recorded included signalment, duration of clinical signs prior to diagnosis, neurological examination findings, MRI of the brain, CSF analysis, treatment schedule used, presence of relapses and long-term outcome. Neurological examination on time of admission and 2, 4 and 6 months after diagnosis, was performed by a board certified neurologist or a neurology resident. On the neurological examination, mentation was divided into bright alert responsive (BAR), quiet alert responsive (QAR), obtundation, stupor and coma, representing decreasing mental status in this order. Possible neuroanatomical localisations included forebrain, brainstem or cerebellum. Dogs with vestibular signs attributable to a brainstem-associated lesion were diagnosed with central vestibular disease. If 2 or more of the above mentioned neuroanatomical regions appeared to be affected on the neurological examination, dogs were given a multifocal neuroanatomical localisation, where dogs with only one region affected were given a focal neuroanatomical localisation. Long-term outcome data were collected through revision of medical records or by contacting the owner or referring veterinarian by email or telephone in September 2016.

Diagnostic investigations

MRI was performed under general anaesthesia with a permanent 0.2T magnet (Airis Mate, Hitachi Ltd, Tokyo, Japan) and included a minimum of T2W and T1W images in a sagittal and transverse plane, and transverse FLAIR images of the entire brain. T1WI were acquired before and after administration of paramagnetic contrast (Dotarem, Guerbet, Brussels, Belgium). Variables recorded were presence of mass effect (brain herniation, midline shift, flattening of gyri/sulci). MR imaging was repeated 4 months after diagnosis following the same protocol, and images were reviewed for evolution of the previously visible lesion(s) (improved, static, or deteriorated).

Parameters analysed in the CSF included TNCC, WBC differentiation and TP concentration. TNCC was considered normal if the total WBC count revealed a number <5 WBC/mm^3. TP concentration was considered normal if <0.25g/l in a cisternal tap and if <0.4g/l in a lumbar tap (Dewey and Da Costa, 2016). TNCC was performed manually using a counting chamber. WBC differentiation was additionally performed manually on the CSF samples with a pleocytosis. For all dogs, PCR examination was performed on CSF for detection of *Bartonella* spp., *Borrelia burgdorferi* sensu latu, canine distemper virus, *Cryptococcus neoformans*, *Cryptococcus gatii*, *Neospora* spp. and *Toxoplasma gondii*.

Treatment, follow-up and outcome

After a presumptive diagnosis of MUA was made, dogs were randomly assigned to 1 of 2 treatment protocols in a 1-2-1-2 way. Protocol 1 consisted of a tapering prednisolone treatment schedule; protocol 2 consisted of the same prednisolone schedule with ciclosporine added to the protocol (**table 5.1**).

***Table 5.1**: Description of two evaluated treatment protocols*.

Protocol 1: Sole prednisolone	**Protocol 2**: Prednisolone + ciclosporine
1.5mg/kg q12h for 3 weeks	+ 5mg/kg ciclosporine q24h
1mg/kg q12h for 6 weeks	+ 5mg/kg ciclosporine q24h
0.5mg/kg q12h for 3 weeks	+ 5mg/kg ciclosporine q24h
0.5mg/kg q24h for 3 weeks	+ 5mg/kg ciclosporine q24h
0.25mg/kg q24h for 3 weeks	+ 5mg/kg ciclosporine q48h
0.25mg/kg q48h	Only 5mg/kg ciclosporine q48h

Neurological examination and CSF analysis were repeated 2, 4 and 6 months after diagnosis, MRI of the brain 4 months after diagnosis. A relapse was defined as a sudden deterioration after initial improvement, mainly consisting of recurrence of the initial or additional neurological signs. If a relapse was noted clinically, the prednisolone schedule was restarted as a rescue protocol. Follow-up information was retrieved from the clinical program or through telephone contact with the owner or referring veterinarian in September 2016.

Statistical Analysis

Data analysis was performed with the aid of a standard statistical software package (Prism, Graphpad Software Inc, La Jolla, California, USA). A Mann-Whitney *U* test was used to compare age, duration of clinical signs prior to diagnosis, TNCC and TP concentration in CSF with long-term outcome. A fisher's exact test was used to compare differences in long-term outcome for presence of seizures and abnormal mentation, neuroanatomical localisation (focal versus multifocal, and focal forebrain versus focal brainstem) and imaging findings (mass effect, foramen magnum herniation and loss of cerebral sulci). Numeric variables were expressed as median and IQR. Values of $P<0.05$ were considered significant.

Results

Signalment and clinical presentation

Fifteen dogs were included in the study. However, three dogs had to be excluded because of a necropsy diagnosis of intracranial lymphoma (n=2) or because of lack of owner compliance to the treatment plan (n=1). For the remaining 12 dogs, breeds included Chihuahua (n=4), Pug dog (n=3), Yorkshire Terrier (n=2), and 1 each of the following breeds: Lhasa Apso, Shih Tzu and Maltese terrier. Nine dogs (75%) were female (of which 3 were neutered) and 3 dogs (25%) were entire males. Median weight at time of diagnosis was 4.45kg

(ranging from 1.75 tot 8.6kg). Median age at time of diagnosis was 28 months (ranging from 11 to 78 months). Median duration of clinical signs prior to diagnosis was 14 days (ranging from 1 to 30 days).

Diagnostic investigations at time of diagnosis

On neurological examination, 2 dogs (17%) were BAR, 3 dogs (25%) were QAR, 6 dogs (50%) were obtunded and 1 dog (8%) was stuporous. Four dogs (33%) had generalized tonic-clonic epileptic seizures of which 1 dog (25%) presented with cluster seizures. Nine dogs (75%) were diagnosed with a focal and 3 dogs (25%) with a multifocal neuroanatomical localisation. Of the dogs with the focal localisation, 4 dogs (44%) presented with a central vestibular lesion and 4 dogs (44%) with a focal forebrain lesion.

As required by the inclusion criteria, MRI of the brain was available for all dogs, revealing multifocal or diffuse lesions affecting the forebrain, brainstem and/or cerebellum. Mass effect was noted in 11 dogs (92%), including loss of cerebral sulci in 7 dogs (64%) and foramen magnum herniation in 4 dogs (36%) (**Figure 5.1**).

Cisternal CSF was obtained in all dogs at time of diagnosis, revealing a normal TNCC in one dog (8%), and an increased TNCC in the remaining eleven dogs (92%), with a median of 283 WBC/mm^3 (ranging from 0-1413.5 WBC/mm^3). Blood contamination was present in two dogs (16%). Manual differentiation mainly revealed a mononuclear pleocytosis. Protein concentration was measured in all samples, and was elevated in all but 1 sample. The median TP concentration was 0.92g/l (ranging from 0.22-17.20g/l). As required by the inclusion criteria, infectious disease testing was negative in all CSF samples. An overview of the clinical and serial CSF findings can be consulted in **table 5.2**.

Diagnostic investigation during treatment

Seven dogs (58%) were alive at the 2-month re-examination, and 5 dogs (42%) at the 4-month and 6-month re-examination.

In the 7 dogs that were alive at the 2-month re-examination, the neurological examination revealed no abnormalities in 4 dogs (57%), residual central vestibular signs in 2 dogs (29%) initially diagnosed with a multifocal lesion, and a mild residual head tilt in 1 dog (14%) initially diagnosed with a central vestibular lesion.

In 5 dogs (42%), follow-up MRI 4 months after diagnosis was available. This revealed an improvement of the lesions in 4 dogs (80%), and resolution of all visible lesions in 1 dog (20%) (**Figure 5.2**).

Follow-up CSF analysis was performed after 2 months in 7 dogs, and after 4 and 6 months in 5 dogs. Results of serial neurological examinations, CSF analysis and imaging findings can be consulted in **table 5.2**.

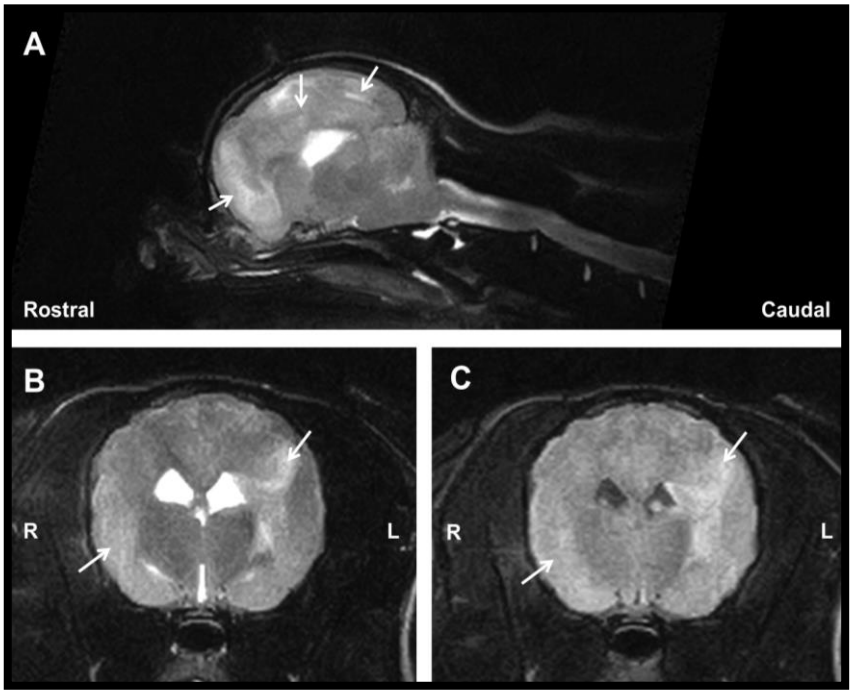

***Figure 5.1**: MRI images of a 17-month-old female entire Pug, presenting with seizures and abnormal mentation, histopathologically diagnosed with NME. Sagittal (A), transverse (B) T2WI and transverse FLAIR (C) images at the level of the interthalamic adhesion revealed presence of a diffuse hyperintense lesion affecting the frontal, parietal, temporal and occipital lobes (white arrows). Loss of cerebral sulci is visible in figures B and C.*

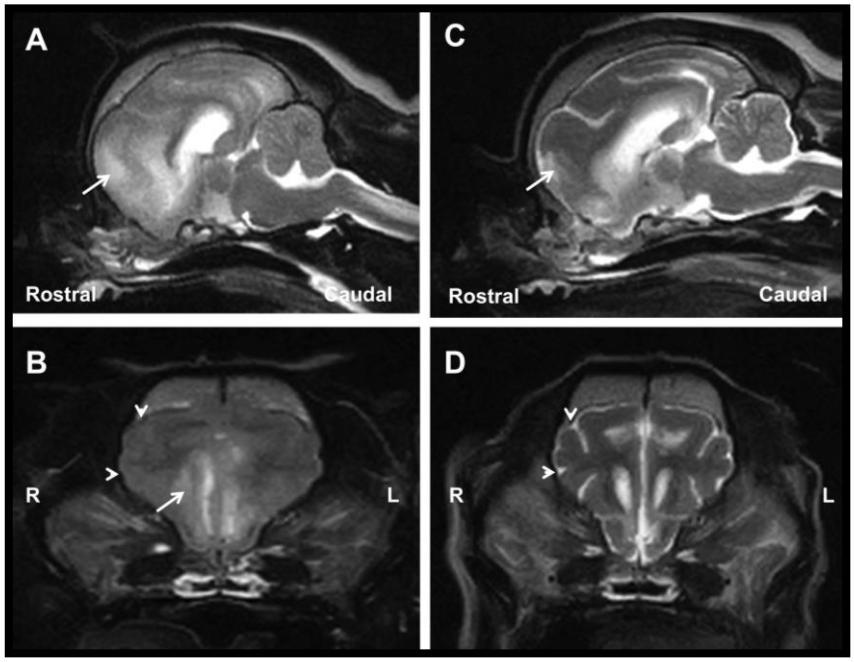

***Figure 5.2**: MR images of a 32-month-old female neutered Pug dog presenting with cluster seizures. Sagittal (A and C) and transverse (B and D) T2WI at the level of the frontal lobe are presented, taken at time of diagnosis (A and B) and 4 months into treatment (C and D). The lesions present in the frontal lobe (white arrows) improved on the follow-up images, as well as the loss of cerebral sulci (arrowheads) noticed on the initial images.*

Treatment and outcome

All dogs received a tapering prednisolone schedule starting with 1.5mg/kg q12h at time of diagnosis. Seven dogs were receiving tapering prednisolone only, ciclosporine therapy was added to the schedule in 5 dogs (41%), and phenobarbital in 4 dogs (33%).

Eight dogs improved on the initial therapy (4 dogs on monotherapy prednisolone, and 4 dogs receiving combination therapy with ciclosporine), 1 dog remained neurologically stable, and 3 dogs deteriorated (2 dogs on monotherapy prednisolone, and 1 dog on combination therapy with ciclosporine). At time of data capture, 3 dogs (25%) were still alive with survival times ranging from 365 to 820 days. These dogs were clinically normal according to the owner, and currently not receiving any medication apart from phenobarbital therapy in 1 dog. Eight dogs (67%) died or were euthanized because of MUA (survival times ranging from 1-1020 days), 1 dog died in a road traffic accident 30 days after diagnosis and this dog was censored for survival analysis. Post mortem examination was only available in 2 of the 8 deceased dogs, revealing NME (both pug dogs). No side effects were noted in any of the dogs treated with ciclosporine. Information regarding relapse and long-term survival time can be consulted in **table 5.2**.

Relapses were observed in 5 dogs (41%), including one relapse in 3 dogs (60%) and two relapses in the remaining 2 dogs (40%). Relapses were seen at a median of 95 days after diagnosis (ranging from 29-138 days). Two dogs were euthanized after diagnosis of the second relapse, the remaining 3 dogs improved neurologically after initiation of the rescue protocol.

The MST was 87 and 567.5 days for dogs treated with sole prednisolone and combination therapy, respectively. However, survival curves were not significantly different between both groups (P=0.49) (**Figure 5.3**). Duration of clinical signs prior to diagnosis (P=0.36), presentation within one week after onset of clinical signs (P=0.55), age at time of presentation (P=0.43), presence of seizures (P=0.21) or abnormal mentation (P=0.99) at time of

diagnosis, presence of focal versus multifocal neurological signs at time of diagnosis (P=0.67), presence of focal forebrain versus focal brainstem (central vestibular disease) at time of diagnosis (P=0.33), and TNCC (P=0.29) and TP concentration (P=0.99) on CSF analysis at time of diagnosis were all not associated with survival. Additionally, the presence of abnormal CSF TNCC at time of diagnosis or re-examination was not associated with relapse (P=0.99). Presence of mass effect (P=0.67), loss of cerebral sulci (P=0.99) or presence of foramen magnum herniation (P=0.67) on MRI were not associated with survival.

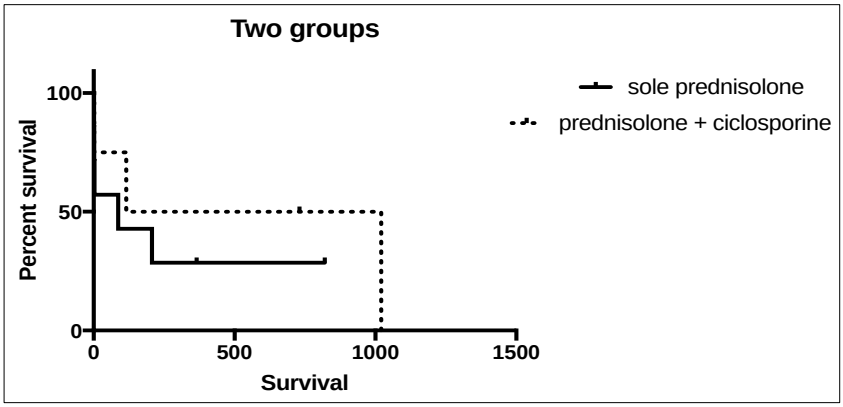

***Figure 5.3**. Kaplan-Meier survival curve comparing the percentage of survival in dogs treated with sole prednisolone therapy (full line) and dogs that received combination therapy of prednisolone and ciclosporine (dotted line). Results were censored for dogs that were still alive at time of data capture (single little blocks).*

***Table 5.2**: Overview of the diagnostic, therapeutic and outcome findings in 12 dogs with MUA. Values above reference limits are marked in bold; Time: 0m = time of diagnosis, 2m = 2 months after diagnosis, 4m = 4 months after diagnosis, 6m = 6 months after diagnosis; RTA = Road Traffic Accident.*

Case	Breed	Time	Relapse (after # days)	TNCC (WBC/ mm^3)	TP (g/l)	Mass effect MRI	Loss cere-bral sulci MRI	Foramen magnum herniation MRI	Ciclo-sporine	Death because of MUA	ST (days)
1	Yorkshire terrier	0m		0	**0.345**	Yes	No	Yes	No	Yes	1
2	Chihuahua	0m		**1313**	**2.556**	No	No	No	No	Yes	3
3	Pug	0m		**46.57**	**0.98**	Yes	Yes	No	No	Yes	207
		2m		**16**	**0.373**						
			93								
		4m		2.75	**0.474**						
			138								
		6m		**8.75**	**0.468**						

Case	Breed	Time	Relapse (after # days)	TNCC (WBC/ mm^3)	TP (g/l)	Mass effect MRI	Loss cere-bral sulci MRI	Foramen magnum herniation MRI	Ciclo-sporine	Death because of MUA	ST (days)
4	Shih Tzu	0m		**957**	**0.369**	Yes	Yes	No	Yes	Yes	1020
		2m		**38.5**	0.209						
			95								
		4m		**59**	**2.434**						
		6m		**11**	**0.252**						
5	Pug	0m		**224.13**	**1.3**	Yes	No	No	Yes	Yes	115
		2m		**15.12**	**0.502**						
			81								
			115								
6	Pug	0m		**316**	**1.11**	Yes	No	No	No	Yes	87
			29								

Case	Breed	Time	Relapse (after # days)	TNCC (WBC/ mm^3)	TP (g/l)	Mass effect MRI	Loss cere-bral sulci MRI	Foramen magnum herniation MRI	Ciclo-sporine	Death because of MUA	ST (days)
		2m		**16.5**	**0.284**						
			87								
7	Maltese terrier	0m		**49.5**	**0.413**	Yes	No	No	No	No	820
		2m		0	0.157						
		4m		0	**0.413**						
		6m		0	0.1						
8	Lhasa Apso	0m		**1413.5**	**17.208**	Yes	Yes	Yes	Yes	Yes	2
9	Chihua-hua	0m		**756**	**1.828**	Yes	No	Yes	Yes	No	730
		2m		**23**							
		4m		**190**							

Case	Breed	Time	Relapse (after # days)	TNCC (WBC/ mm^3)	TP (g/l)	Mass effect MRI	Loss cere-bral sulci MRI	Foramen magnum herniation MRI	Ciclo-sporine	Death because of MUA	ST (days)
			120								
		6m		0	0.177						
10	Yorkshire terrier	0m		**77**	0.229	Yes	Yes	No	No	Yes	2
11	Chihuahua	0m		**420**	**0.288**	Yes	Yes	Yes	Yes	Not related (RTA)	30
12	Chihuahua	0m		**250**	**0.879**	Yes	Yes	No	No	No	365
		2m		0	**0.364**						
		4m		**1551**	**2.127**						
			126								
		6m		**26**	**0.323**						

Discussion

This study evaluated and provided two treatment regimens for dogs with MUA, and evaluated possible prognostic factors for survival. Although it was hypothesized that combination therapy would result in improved survival compared to sole prednisolone therapy, no significant difference in MST could be detected, most likely caused by the low case number. Additionally, previously established prognostic factors could not be confirmed in the present study.

Ciclosporine therapy has previously been described as sole treatment for MUA (Adamo and O'Brien, 2004; Adamo et al., 2007), or as combination therapy with prednisolone (Adamo and O'Brien, 2004; Gnirs, 2006; Adamo et al., 2007; Jung et al., 2007; Pakozdy et al., 2009; Kang et al., 2009; Jung et al., 2012; Jung et al., 2013), ketoconazole (Adamo et al, 2007), or cytosine arabinoside and prednisolone (Behr et al., 2009). Twenty-six dogs have been reported receiving prednisolone doses ranging from 2-30mg/kg/d combined with ciclosporine doses ranging from 6-30mg/kg/d (Adamo and O'Brien, 2004; Gnirs, 2006; Adamo et al., 2007; Jung et al., 2007; Pakozdy et al., 2009; Kang et al., 2009; Granger et al., 2010; Jung et al., 2012; Jung et al., 2013). In the presented study, a more immunosuppressive prednisolone schedule as previously described by Talarico and Schatzberg (2010) was used, combined with 5mg/kg/d of ciclosporine. This is the approved dose for atopic dermatitis in dogs (a common pruritic dermatologic problem affecting dogs, associated with IgE antibodies targeting environmental antigens). The same dose was advised in dogs with pemphigus foliaceus, an autoimmune skin disease. It was however anecdotally reported that dogs receiving this lower dosages of ciclosporine (≤5mg/kg q24h) can develop severe secondary infections (Archer et al., 2014). On the contrary, higher doses of ciclosporine (5-10mg/kg q12h) have been advised in dogs with acute and possibly life-threatening auto-immune diseases, including immune-mediated thrombocytopenia and immune-mediated haemolytic anaemia, to achieve clinically relevant depression of T-lymphocytes and as such immunosuppression (Archer et al., 2014). Therefore, we wanted to investigate whether this lower dose of ciclosporine combined with an

immunosuppressive schedule of prednisolone would be of additional value in dogs with MUA, which we could not prove in the presented study.

The discrepancy in number of dogs included in both treatment groups (7 dogs were treated with sole prednisolone therapy and 5 dogs with combination therapy with ciclosporine) is mainly caused by the 3 cases that were initially included in the study and that were mainly addressed to the combination therapy group. After exclusion, there was no equal randomization between groups.

None of the previously established prognostic factors for dogs with MUA, including age and presence of seizures or altered mentation at time of diagnosis, presentation within 7 days after onset of clinical signs, abnormal CSF analysis at time of diagnosis, and presence of mass effect, foramen magnum herniation or loss of cerebral sulci on MRI, could be confirmed in the presented study. Munana and Luttgen (1998) found significant longer survival times for dogs with focal neurological signs versus dogs with multifocal neurological signs. Additionally, dogs with focal forebrain signs had a significantly longer survival time compared to dogs with focal signs related to other areas of the CNS. This finding was not repeated in more recent studies (Coates et al., 2007; Lowrie et al., 2013) nor in the presented study.

One study identified a lower CSF TNCC to be significantly associated with improved survival in dogs with MUA (Oliphant et al., 2016), whilst others found that neither CSF TNCC nor TP concentration had an effect on survival time in dogs with MUA (Coates et al., 2007). The study of Lowrie et al. (2013) failed to demonstrate an association between normal CSF analysis and improved outcome, but did find an association between abnormal CSF analysis at three months and relapse or poor outcome (Lowrie et al., 2013). In the study of Mercier and Barnes Heller (2015) CSF analysis was repeated 1 month after diagnosis, and their results suggested that serial CSF analysis might be a valid tool for monitoring success or failure of treatment in dogs diagnosed with MUA and treated with glucocorticoid monotherapy. In the presented study however, TNCC and TP of CSF were not predictive for outcome or relapse.

Overall, 26 dogs have been previously reported receiving initial doses of ciclosporine ranging from 3-15mg/kg PO every 12 hours, resulting in MSTs ranging from 236 to 930 days (Adamo and O'Brien, 2004; Gnirs, 2006; Adamo et al., 2007; Jung et al., 2007; Pakozdy et al., 2009; Kang et al., 2009; Jung et al., 2013). One dog receiving combination therapy survived for 1096 days (Jung et al., 2012). In the presented study, the MST was 87 and 567.5 days for dogs treated with sole prednisolone and combination therapy, respectively, however survival curves were not significantly different between both groups. Overall, only 2 previous studies revealed a mean ST of 305 days (Jung et al., 2007) or a MST of 602 days (Pakozdy et al., 2009) for dogs treated with ciclosporine and prednisolone, which is comparable to the MST of 567.5 days calculated in the presented study. It is however important to state that both studies are performed with a comparable small number of dogs, and a similar ciclosporine dose (6mg/kg/day for the study of Pakozdy et al. (2009) and 5mg/kg/d for the study of Jung et al. (2007)). On the contrary, the initial doses of steroids used in these studies differ from the initial dose used in the presented study (3mg/kg/d), being 5-30mg/kg/d and 2mg/kg/d, respectively. Survival times on combination therapy in the presented study ranged from 2 – 1020 days, which is comparable to the 2 – 1096 days reported in literature (Adamo et al., 2007; Kang et al., 2009; Jung et al., 2012; Jung et al., 2013).

Results of the presented study should however be interpreted with caution, mainly because of the small sample size and the limited number of dogs that was alive at time of data capture (1 of the 4 dogs alive was censored for survival analysis). Definitive histopathology is lacking in all but 2 cases, revealing NME. Interestingly, all dogs included in the study can be considered breeds that are predisposed to develop NE including Pug, Yorkshire Terrier, Maltese, Chihuahua, Pekingese, Papillon, Shih Tzu, Coton de Tulear and Brussels Griffon (Talarico and Schatzberg, 2010; Cooper and others, 2014). It is however stated that dogs of any breed and age can be affected by all subtypes of MUA (Granger et al., 2010; Coates and Jeffery, 2014). Additionally, one dog with a normal TNCC was included in the study. Normally, a pleocytosis (>5 WBC/mm^3) with monocytic predominancy is one of the inclusion criteria for MUA in dogs. On the contrary, it is previously stated that CSF examination can

be normal in 3-57% of dogs with MUA (Menaut et al., 2009; Granger et al., 2010), in 16% of dogs with GME and in 12.5% of dogs with NE (Granger et al., 2010). Albuminocytological dissociation has been found in cases with a normal cell count (Granger et al., 2010). The included dog was a 78-month-old male neutered Yorkshire terrier with multifocal lesions mainly affecting the subcortical white matter, and so a presumptive diagnosis of NLE was suspected and the dog was included in the study.

A larger prospective, multi-centre study should be performed including more dogs to establish the previously published prognostic factors and the here described treatment protocol.

Conclusions

In the presented study, 67% of dogs died because of MUA with no difference in outcome seen between dogs treated with sole prednisolone therapy and combination therapy with ciclosporine. Overall, initiation of treatment resulted in a MST of 115 days. Several described prognostic factors to select for optimal treatment candidates and to manage owner expectance, could not be confirmed. Further studies including more cases are warranted to evaluate the efficacy of the two described treatment protocols.

Part III

Prognostic Factors and Short-term Outcome

Chapter 5

PROGNOSTIC FACTORS FOR ONE-WEEK SURVIVAL IN DOGS DIAGNOSED WITH MENINGOENCEPHALOMYELITIS OF UNKNOWN AETIOLOGY

Ine Cornelis[a], Holger A. Volk[b], Luc Van Ham[a], Steven De Decker[b]

[a]*Small Animal Department, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium.*
[b]*Clinical Science and Services, The Royal Veterinary College, University of London, Hatfield, United Kingdom.*

Adapted from: Cornelis I, Volk HA, Van Ham L, De Decker S (2016). Prognostic factors for one-week survival in dogs diagnosed with meningoencephalomyelitis of unknown aetiology. *The Veterinary Journal* 214, 91-95.

Abstract

Although several studies have evaluated the long-term outcome of MUA in dogs, little is known about short-term survival and initial response to therapy. The aim of this study was therefore to evaluate possible prognostic factors for 7-day survival after diagnosis of MUA in dogs.

Medical records were reviewed for dogs diagnosed with MUA between 2006 and 2015. Previously described inclusion criteria were used, and for all dogs 7-day survival data needed to be available. A poor outcome was defined as death within 1 week. One hundred and sixteen dogs met the inclusion criteria. Thirty dogs (26%) died within 7 days after making a presumptive diagnosis of MUA. Assessed variables included age, sex, weight, duration of clinical signs and treatment prior to diagnosis, venous blood glucose and lactate levels, white blood cell count on complete blood count, total nucleated cell count / total protein concentration / white blood cell differentiation on cerebrospinal fluid analysis, presence of seizures and cluster seizures, mentation at presentation, neuroanatomical localisation, imaging findings and treatment after diagnosis. Univariate statistical analysis was performed to identify variables to be included in a multivariate model. Multivariate analysis identified three variables significantly associated with poor outcome, including decreased mentation at presentation, presence of seizures, and increased percentage of neutrophils on cerebrospinal fluid analysis.

Despite initiation of appropriate treatment, more than a quarter of dogs died within one week after making a presumptive diagnosis of MUA, emphasizing the need for evaluation of short-term prognostic factors. The information of this study can aid in the management of expectations of both clinical staff and owners with dogs diagnosed with MUA.

Introduction

MUA describes all clinically diagnosed cases of GME, NME and NLE that lack histopathological confirmation (Coates and Jeffery, 2014). A clinical diagnosis can be achieved based on a combination of neurological examination results, MRI findings and CSF abnormalities (Coates and Jeffery, 2014). The exact aetiology and pathophysiology of MUA are currently unknown, but the cornerstone of medical treatment is immunosuppressive therapy. Several treatment protocols using different immunomodulating drugs, resulting in different long-term survival times have been reported (Sisson et al. 1989; Gregory et al., 1998; Munana and Luttgen, 1998; Adamo and O'Brien, 2004; Gnirs, 2006; Zarfoss et al., 2006; Adamo et al., 2007; Coates et al., 2007; de Stefani et al., 2007; Feliu-Pascual et al., 2007; Uriarte et al., 2007; Jung et al., 2007; Menaut et al., 2008; Pakozdy et al., 2009; Smith et al., 2009; Granger et al., 2010; Kang et al., 2009; Wong et al., 2010; Flegel et al., 2011; Jung et al., 2012; Jung et al., 2013; Lowrie et al., 2013; Beckmann et al., 2015; Mercier and Barnes Heller, 2015; Barnoon et al., 2015; Lowrie et al., 2016).

Although several studies have focused on long-term survival, little is known about early survival and initial response to therapy of dogs diagnosed with MUA. The primary aim of this study was therefore to evaluate early survival and initial response to immunosuppressive therapy in those dogs. The secondary aim of this study was to investigate possible prognostic factors for 7-day survival after diagnosis of MUA. It was hypothesized that a substantial part of dogs with MUA would succumb in the first week after diagnosis despite appropriate treatment and monitoring. It was further hypothesized that specific characteristics of the clinical presentation, neurological examination, clinical pathology abnormalities, imaging findings and type of treatment would be associated with 7-day survival in dogs with a presumptive diagnosis of MUA.

Materials and methods

Case selection

The electronic medical database of the Small Animal Referral Hospital, Royal Veterinary College, University of London, was searched between January 2006 and April 2015 for dogs diagnosed with MUA. Dogs were included based on the criteria used by Granger et al. (2010), if they had (1) complete medical records available, (2) a complete neurological examination performed leading to a focal or multifocal intracranial neuroanatomical localisation, (3) inflammatory CSF analysis, (4) MR imaging of the brain demonstrating single, multiple or diffuse intra-axial hyperintense lesions on T2W images, and if (5) 7-day follow-up information was available. Dogs were excluded if (1) the clinical records or imaging studies were incomplete or not available for review, (2) dogs were diagnosed with meningomyelitis without clinical signs of intracranial involvement, (3) no pleocytosis was found on CSF analysis with the exception of dogs with signs of raised ICP on imaging studies, in which case CSF collection was not performed, and if (4) positive infectious diseases titres were found on serology or PCR examination for canine distemper virus, *Toxoplasma gondii* or *Neospora caninum.* Dogs with histopathological confirmation of the disease only needed to fulfil inclusion criteria (1) and (5). Information retrieved from the medical records included breed, gender, age at diagnosis, sex, body weight, results of neurological examination and neuroanatomical localisation, duration of clinical signs and treatment prior to diagnosis, presence of concurrent diseases, results of CBC and biochemistry profile, results of CSF analysis including TNCC, WBC differentiation and TP concentration, lactate and glucose concentration on venous blood gas analysis, treatment received and 7-day survival time.

Dogs were divided in two groups based on their body weight: dogs <15kg, in this paper referred to as small dogs; and dogs >15kg, in this paper referred to as large dogs. Mentation was divided into bright alert responsive (BAR), quiet alert responsive (QAR), obtundation, stupor and coma,

representing decreasing mental status in this order. Possible neuroanatomical localisations included forebrain, brainstem or cerebellum. Dogs with vestibular signs attributable to a brainstem-associated lesion were diagnosed with central vestibular signs. If 2 or more of the above mentioned regions appeared to be affected on the neurological examination, dogs were given a multifocal neuroanatomical localisation, where dogs with only 1 region affected were given a focal neuroanatomical localisation. MRI was performed under general anaesthesia with a permanent 1.5T magnet (Intera, Philips Medical Systems, Eindhoven, the Netherlands) and all images were reviewed by a board certified neurologist (SDD) using Osirix Dicom viewer (Osirix Foundation, V.5.5.2 Geneva, Switzerland). The reviewer was blinded for the results of the neurological examination, outcome after 7 days and histopathological findings. Sequences could vary, but studies included a minimum of T2WI (TR/TE, 3000/120), T1WI (TR/TE, 400/8) and FLAIR images of the entire brain in a sagittal, transverse and dorsal plane. The T1WI were acquired before and after IV administration of paramagnetic contrast medium (0.1mg/kg, gadoterate meglumine, Dotarem, Guerbet, Milton Keynes, UK). Variables recorded were lesion localisation and distribution, presence of parenchymal or meningeal contrast enhancement and presence of mass effect (brain herniation, midline shift, flattening of gyri/sulci). For dogs that had CSF analysis performed, site of collection (cisternal or lumbar), TNCC, TP and cytological differentiation were recorded. Total nucleated cell count was considered normal if the TNCC <5 WBC/mm^3. Total protein concentration was considered normal for a cisternal collection if <0.25g/l and for a lumbar collection if <0.4g/l (Dewey and Da Costa, 2016).

Treatment and follow-up

For all dogs, the specific treatment protocol was recorded (corticosteroids with or without cytosine arabinoside). During hospitalisation, all dogs underwent daily at least one general physical and complete neurological examination by a board-certified neurologist or neurology resident. The results of the neurological examination as well as response to treatment (improvement, deterioration or static) were systematically recorded on the kennel sheets.

Follow-up information for the first 7 days after diagnosis was collected from the medical records. If dogs were discharged within the first seven days, medical records were searched for the presence of a re-examination or the presence of owner communication confirming the dog being alive. Dogs were excluded from the study if this information was not available. A successful outcome was defined as survival for at least 7 days after making a diagnosis of MUA, while an unsuccessful outcome was defined as death in the first 7 days after diagnosis. For dogs that died in the first week after diagnosis, it was recorded if dogs were euthanized at owner request after a diagnosis was made and without treatment, if dogs failed to recover from general anaesthesia after MRI, or if dogs died or were euthanized due to progression of disease after successful recovery from general anaesthesia. Dogs that did not survive general anaesthesia or were euthanized at owner request after a diagnosis was made without treatment were not included for further analysis.

Statistical analysis

Outcome was defined as death or alive 7 days after diagnosis. Data analysis was performed with the aid of a standard statistical software package (Prism, Graphpad Software Inc, La Jolla, California, USA). A Mann-Whitney *U* test was used to compare age, weight, duration of clinical signs prior to diagnosis, venous blood glucose and lactate levels, white blood cell (total, neutrophil and lymphocyte) count on CBC, TNCC/TP/neutrophil percentage in CSF, between dogs that were death **or** alive one week after diagnosis. A fisher's exact test was used to compare differences in sex, treatment prior to diagnosis, presence of seizures and cluster seizures, mentation (BAR, QAR, obtundation, stupor, coma), neuroanatomical localisation (multifocal, forebrain, brainstem, central vestibular), treatment after diagnosis (steroids, cytosine arabinoside, mannitol) and imaging findings (lesion localisation, meningeal or parenchymal contrast enhancement, mass effect, brain herniation, flattening gyri/sulci, rostral or caudal transtentorial herniation, foramen magnum herniation) between dogs that were death and alive one week after diagnosis.

A binary response mixed model was carried out using SPSS (Statistical Package for the Social Sciences v. 21.0.1; SPSS Inc.). Whether the dog was dead or alive 7 days after diagnosis was the binary response variable. Factors found to be significant at the univariate level were taken forward for multivariate analysis. Body weight, duration of clinical signs, lactate concentration on venous blood gas analysis, TNCC on CSF analysis and percentage of neutrophils in CSF were modelled as continuous fixed effects. Mentation was modelled as a categorical fixed effect, and the presence of seizures, cluster seizures and cytosine arabinoside administration were modelled as binomial fixed effects. Breed was included as a random effect, with cross breeds coded plainly as 'cross breed' due to the unknown parentage of many of these dogs. This random effect took into account the genetic non-independence of multiple members of the same breed in the study population, and possible demographic and environmental factors. Multicollinearity was checked for in all models, identified from inflated standard errors in the models, and thus avoided. Model fit was assessed using the deviance and Akaike's information criterion. Numeric variables were expressed as median and IQR. Values of $P<0.05$ were considered significant. Receiver operating characteristic (ROC) analysis was performed to examine the performance of the significant continuous variables on multivariate analysis as an indicator of prognosis, by determining the power of the test by measuring the area under the curve (AUC). A perfect test has an AUC value of 1.0, with an AUC of 0.5 means the test performs no better than chance.

Results

Signalment

One hundred and sixteen dogs met the inclusion criteria and were included in the study. Eighty-seven small dogs (75%) and 29 large dogs (25%) were included in the study. Median age at time of presentation was 52.5 months (4 – 146 months) and median body weight was 9.2kg (1.65-94 kg). Fifty dogs (43%) were female, of which 30 were neutered, compared to 66 males (57%),

of which 40 were neutered. Median duration of clinical signs prior to diagnosis was 7 days (range 1-180 days). Twenty dogs (17%) were treated with anti-inflammatory doses of glucocorticosteroids (dose ranging from 0.5-1mg/kg every 12-24 hours) prior to diagnosis, with a median duration of 3.5 days (ranging from 1-90 days).

Neurological examination

Mentation was classified as BAR in 30 dogs (26%), QAR in 21 dogs (18%), obtundation in 59 dogs (51%) and stupor in 6 dogs (5%). No dogs presented comatose. Twenty-nine dogs (25%) presented with seizures, of which 20 dogs (69%) presented with cluster seizures and 2 dogs (31%) with status epilepticus. Sixty-six dogs (57%) presented with multifocal neurological signs, 50 dogs (43%) with focal neurological signs. Of those 50 dogs, 39 dogs (78%) presented with focal forebrain signs, 8 dogs (16%) with focal brainstem signs, 2 dogs (4%) with focal cerebellar signs, and 1 dog (2%) with central vestibular signs.

Diagnostic findings

Results of CBC and biochemistry profile were available in 97 dogs (84%). Leucocytosis was present in 13 dogs (13%) and lymphopenia in 32 dogs (33%). Serology and/or PCR analysis for *Toxoplasma gondii, Neospora caninum* and canine distemper virus were available and negative in 82 dogs (71%). Lactate and glucose concentrations on venous blood gas analysis were available in 49 dogs (42%), revealing an increased lactate and/or glucose concentration in 9 (18%) and 12 (24%) dogs, respectively. CSF analysis was not performed in 20 dogs (17%), revealed no abnormalities in 3 dogs (3%) and a pleocytosis in the remaining 93 dogs (80%). In the 3 dogs with a normal TNCC, complete necropsy revealed GME (n=1), NME (n=1) or NLE (n=1). For the dogs where a pleocytosis was found (n=93), median TNCC was 80 WBC/mm^3 (ranging from 6-2560 WBC/mm^3). For the dogs that died in the first week after diagnosis, median percentage of lymphocytes, neutrophils and

monocytes/macrophages was 54%, 5% and 24%, respectively, compared to dogs that survived the first week after diagnosis where percentages were 66%, 1% and 23%, respectively. Pre-treatment with glucocorticosteroids did not significantly influence the TNCC on CSF analysis (P=0.9116).

Magnetic resonance imaging revealed a focal lesion in 31 dogs (27%), a multifocal lesion in 77 dogs (66%) and a diffuse lesion in 8 dogs (7%). Mass effect was seen in 66 dogs (57%), consisting of brain herniation (n=44), midline shift (n=38) and/or flattening of gyri or sulci (n=51).

Treatment and outcome

All but two dogs were alive after MR imaging. Spontaneous breathing did not return in one dog (1%) after anaesthesia, treatment was initiated with dexamethasone but the dog was euthanized after 1 hour at owners request. As only an attempt of 1 hour was made, this dog was excluded for further analysis. One dog (1%) was euthanized during general anaesthesia at owners request because of severe neurological signs, without an attempt for treatment. The remaining 114 dogs (98%) were treated with glucocorticosteroids. Detailed treatment data were only available in 104 cases, and treatment consisted mainly of an intravenous (IV) dose of dexamethasone within hours after reaching a diagnosis (dose ranging from 0.3-0.6mg/kg), followed by oral prednisolone (dose ranging from 1-2mg/kg every 12-24 hours) therapy (n=79), or of oral prednisolone therapy (dose ranging from 1-2mg/kg every 12-24 hours) that was initiated within hours after diagnosis (n=25). Eighty-eight of 114 dogs (85%) received additional treatment with cytosine arabinoside. This was given as subcutaneous (SC) injections (50mg/m^2 SC every 12 hours for 2 consecutive days) in 69 dogs (78%) and as an IV constant rate infusion (CRI) (200mg/m^2 over 8 hours) in 19 dogs (22%). Twenty-seven dogs (23%) required mannitol (0.5-1g/kg IV over 15-20 minutes) administration during hospitalisation for clinical signs suggestive of raised ICP. This was administered immediately after intracranial MRI in 9 dogs (33%) and during hospitalisation in the remaining 18 dogs (67%) at a median time after diagnosis of 1 hour (ranging from 1-48 hours).

Of the 114 dogs in which treatment was initiated, 84 (74%) survived and, 30 dogs (26%) died or were euthanized during the first 7 days after making a presumptive diagnosis of MUA. These dogs died (n=10) or were euthanized (n=20) due to deteriorating neurological signs. A median survival time (MST) of 1 day was calculated for all dogs that died within one week after diagnosis. Overall, histopathological confirmation (necropsy) was available in 14 dogs, revealing a diagnosis of GME (n=9), NME (n=4) or NLE (n=1). Dogs that demonstrated neurological improvement did so within a median time of 24 hours after diagnosis (ranging from 12-72 hours) and clinical improvement within this time period was significantly associated with 7-day survival (P<0.0001).

Factors associated with survival

Univariate analysis revealed that higher body weight (P=0.027), shorter duration of clinical signs prior to diagnosis (P=0.042), decreased mentation at presentation (P=0.048), presence of seizures (P=0.0015) or cluster seizures (P=0.0050), increased lactate concentration on venous blood gas analysis (P=0.026), higher TNCC on CSF analysis (P=0.031), higher percentage of neutrophils in CSF (P=0.0224), administration of IV dexamethasone (P=0.0019), and no administration of cytosine arabinoside (P=0.012), all were associated with a poor outcome. Administration of a cytosine arabinoside CRI was significantly associated (P<0.0001) with a poor outcome compared to administration of subcutaneous (SC) cytosine arabinoside. None of the other evaluated clinical, clinical pathology, or imaging variables were significantly associated with outcome in this model (**Table 6.1**).

***Table 6.1**: Results after univariate analysis. Values are numbers with respective percentages or median values with respective interquartile ranges. Values differ significantly at P<0.05 (marked with bolt text and *). Dogs (n=2) that did not recover from the general anesthesia for MR imaging, were not included in the analysis considering treatment.*

Variable	Death ≤7 days (n=32)	Alive after 7 days (n=82)	P value *
Signalment			
Age (months)	55 (7 - 35)	50.5 (4 - 146)	0.987
Male	21 (66%)	45 (54%)	0.521
Female	11 (34%)	39 (46%)	0.521
Body weight (kg)	**10.25 (3 – 94)**	**8.9 (1.65 – 54.9)**	**0.027***
Duration of clinical signs prior to diagnosis (days)	**6 (1 – 60)**	**8 (1 – 180)**	**0.042***
Treatment with glucocorticosteroids prior to diagnosis (days)	1.5 (1 – 9) (n=4)	3 (1 – 48) (n=16)	0.061
Clinical signs			
Seizures	**15 (47%)**	**14 (17%)**	**0.0015***
Cluster seizures	**11 (34%)**	**9 (11%)**	**0.0050***
Neuroanatomical localisation			
Forebrain	24 (75%)	56 (67%)	0.502
Brainstem	21 (66%)	50 (60%)	0.671
Central vestibular	8 (25%)	23 (27%)	1.000

Variable	Death ≤7 days (n=32)	Alive after 7 days (n=82)	P value *
Abnormal mentation	7 (22%)	23 (27%)	0.362
Stuporous	**4 (13%)**	**2 (2%)**	**0.048***
Complete blood count	27 (84%)	70 (85%)	
White blood cells (.10^9/l)	13.10 (3.54 – 25.1)	9.97 (4.6 – 32.8)	0.103
Neutrophils (.10^9/l)	10.16 (2.4 – 23.9)	7.2 (3 – 28.3)	0.267
Lymphocytes (.10^9/l)	1.1 (0.1 – 3.5)	1.3 (0.17 – 3.6)	0.177
Lymphopenia	11 (34%)	21 (25%)	0.217
Venous blood gas	15 (47%)	34 (41%)	
Lactate (mmol/l)	**2.1 (0.5 – 5.5)**	**1.4 (0.4 – 5,6)**	**0.026***
Glucose (mmol/l)	6.3 (4 – 7.9)	5.69 (3.2 – 11.1)	0.100
CSF analysis	25 (78%)	69 (84%)	
TNCC (WBC/mm^3)	**364 (1 – 2220)**	**66 (5 - 2560)**	**0.031***
Total protein (g/l)	0.79 (0.1 – 5.56)	0.46 (0.11 – 8.5)	0.410
Not performed because signs of raised ICP	7 (22%)	13 (15%)	0.289
Lymphocyte percentage	54 (2 – 97)	66 (1 – 98)	0.0874
Neutrophil percentage	5 (0 – 64)	1 (0 – 61)	**0.0224***
Monocyte/macrophage percentage	24 (3 – 87)	23 (0 – 92)	0.981
MRI findings			

Variable	Death ≤7 days (n=32)	Alive after 7 days (n=82)	P value *
Focal lesion	7 (22%)	24 (29%)	0.635
Multifocal lesion	22 (69%)	55 (65%)	0.635
Diffuse lesion	3 (9%)	5 (6%)	0.386
Forebrain localisation	26 (81%)	63 (75%)	0.327
Brainstem localisation	16 (50%)	45 (54%)	0.445
Cerebellum localisation	4 (13%)	17 (20%)	0.248
Mass effect	16 (50%)	50 (60%)	0.405
Brain herniation	8 (25%)	36 (43%)	0.058
Caudal transtentorial	7 (22%)	36 (43%)	0.053
Foramen magnum	8 (25%)	22 (26%)	0.549
Midline shift	11 (34%)	27 (32%)	0.492
Flattening gyri/sulci	15 (47%)	36 (43%)	0.427
Contrast enhancement			
Meningeal	24 (75%)	54 (64%)	0.191
Parenchymal	20 (63%)	60 (71%)	0.239
Treatment			
Dexamethasone	**27 (84%)**	**52 (62%)**	**0.0019***
Prednisolone	**1 (6%)**	**24 (28%)**	**0.0019***
Cytosine arabinoside	**19 (60%)**	**69 (82%)**	**0.012***
CRI	**12 (20%)**	**7 (10%)**	**< 0.0001***

Variable	Death ≤7 days (n=32)	Alive after 7 days (n=82)	P value *
SC	**9 (47%)**	**60 (87%)**	**< 0.0001***
Mannitol	8 (25%)	19 (23%)	0.806
Improvement after treatment	**4 (13%)**	**82 (97%)**	**<0.0001***
Time from diagnosis to treatment with corticosteroids (hours)	2 (1 – 48)	2 (1 – 72)	0.153

A binary response mixed model was carried out on factors found to be significant at the univariate level. Three variables were significantly associated with poor outcome in the final model: percentage of neutrophils in CSF, decreased mentation at presentation and a history of seizures (**Table 6.2**).

Table 6.2*: Results of binary response mixed model analysis (reference category: dead at 1 week). Values differ significantly at P<0.05 (marked with *). OR = odds ratio, CI = confidence interval, SE = standard error.*

Variable	Sub category	SE (coefficient)	OR (95% CI OR)	t	p
Neutrophils	-	0.96	0.093-0.99	-2.21	**0.030***
Mentation	BAR	18.33	1.39-241.33	2.24	**0.027***
	QAR	4.77	0.41-55.00	1.27	0.208
	Obtundation	6.40	0.58-69.72	1.55	0.126
	Stupor		*Reference category*		
Seizures	No	4.20	1.08-16.37	2.10	**0.039***
	Yes		*Reference category*		

Dogs with a higher % of neutrophils were at an increased risk of death at 1 week (mean ± SE dead: 14.88 ± 4.01; alive: 6.31 ± 1.40), with a negative association between increased % of neutrophils and likelihood of being alive at 1 week. Dogs with a decreased mentation at presentation were at increased risk of death within 1 week (% dead at 1 week BAR: 20% versus stupor: 66.7%), with dogs presented BAR had an 18.33 increased odds of being alive at 1 week compared to those presented in a stuporous state. Finally, dogs with a history of seizures were at an increased risk of death at 1 week (dead at 1 week no seizures: 19.5% vs. seizures: 51.7%), with dogs without seizures had a 4.20 increased odds of being alive at 1 week compared to those with seizures. ROC-analysis revealed that none of the significant continuous variables was able to reliably differentiate between good and poor short-term outcome in dogs with MUA (**Figure 6.1**).

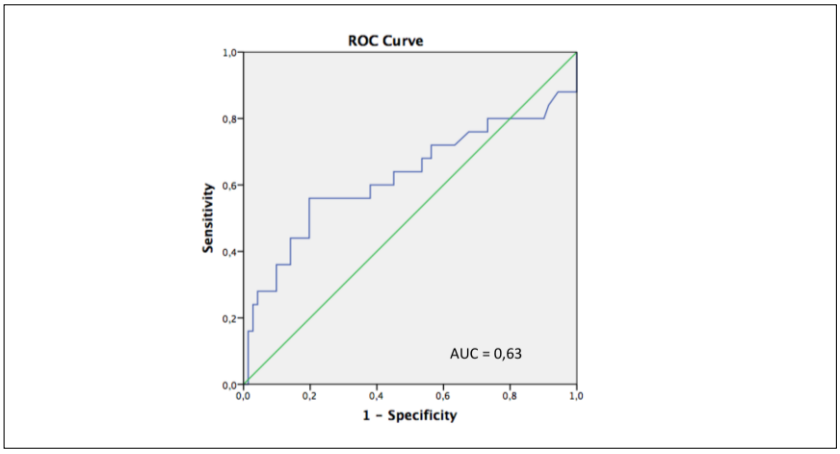

Figure 6.1*: ROC curve for neutrophil percentage in CSF. The AUC was 0.63, indicating that this continuous variable has no clinical use in differentiating between good and poor outcome within 7 days after diagnosis. Consequently, no reliable threshold values with a combined high sensitivity and specificity could be identified to differentiate between both dogs with a good and poor outcome.*

Discussion

This study evaluated the prevalence and potential risk factors for 1-week survival in dogs diagnosed with MUA. Although it was hypothesized that a proportion of dogs would not survive the first week after obtaining a diagnosis of MUA, a high proportion (26%) of dogs died within this specific time period despite initiation of appropriate treatment and close monitoring. Despite the lack of specific studies evaluating short-term survival in dogs with MUA, our findings indicate better outcomes compared with previous findings. It has been stated that approximately 15% of dogs diagnosed with GME died before being treated (Granger et al., 2010), compared to only 1/116 dogs (0.9%) in the presented study where the owner decided to euthanize the dog without attempting to treat. A recent study reported that 56% of dogs diagnosed with and treated for MUA died or were euthanized with a median survival time of 2 days (Lowrie et al., 2013), which is almost double the percentage of the presented study. The results of our study demonstrated that 25% of dogs would die in the first week after diagnosis and initiating appropriate treatment for MUA. It seems therefore important to include this group of dogs when considering the overall prognosis of dogs with MUA.

In a recent study by Sharma and Holowaychuk (2015), increased venous lactate concentrations were a risk factor for non-survival to hospital discharge in dogs with head trauma. Additionally, hyperglycaemia has been associated with severity of injury in cases of head trauma in dogs and cats, but not with outcome (Syring et al., 2001). In the presented study, blood glucose and lactate levels were measured on admission or before MR imaging on standard venous blood gas analysis. No significant difference was found in blood glucose levels between dogs that did or did not survive the first week after diagnosis. In the univariate analysis, lactate concentrations were significantly increased in dogs with a poor outcome, but this result was not confirmed in the multivariate analysis. As both measurements were only available for review in approximately 20% of the cases, further studies should be performed to draw further conclusions.

This study identified some potential risk factors for death in the first 7 days after obtaining a presumptive diagnosis of MUA. In agreement with previous findings, dogs presenting with seizures and/or a decreased mentation were at increased risk of not surviving the first week after diagnosis (Bateman and Parent, 1999; Coates et al., 2007). Although it is possible these dogs represent a group of animals with a worse clinical phenotype, it cannot be excluded that the necessity of administering anti-epileptic drugs in these patients was associated with increased sedation and therefore contributed to a further decline of their neurological function. In contrast to results of a recent study (Lowrie et al., 2013), a higher neutrophil percentage on CSF analysis was significantly associated with an increased risk of death in the first week after a diagnosis of MUA. However, the constructed ROC-curve could not demonstrate a reliable threshold value with a combined high sensitivity and specificity to predict survival and so the exact neutrophil percentage should not be considered a useful tool for assessing prognosis in individual animals with MUA.

In previous studies, it was stated that adding another immunosuppressive agent or radiation therapy to the standard treatment protocol with glucocorticosteroids improves survival of dogs with MUA (Munana and Luttgen, 1998; Jung et al., 2007; Coates et al., 2007; Granger et al., 2010; Flegel et al., 2011; Beckmann et al., 2015; Barnoon et al., 2015) but this could not be confirmed in the present study. Unexpectedly, treatment with SC injections of cytosine arabinoside and oral prednisolone therapy were both significantly associated with a better short-term outcome. Most likely, this is highly biased at the level of the clinician, who would possibly opt for IV dexamethasone and an additional CRI of cytosine arabinoside in dogs with more severe neurological signs. Additionally, a previous study (Crook et al., 2013) indicated more favourable pharmacokinetic properties of intravenous continuous infusion of cytosine arabinoside compared to subcutaneous injections. Both findings could not be confirmed in the multivariate analysis performed in our study.

This study is obviously limited by its retrospective character. Inclusion criteria were based on previously reported studies, but they are not restrictive. In the present study, dogs were excluded if TNCC on CSF analysis and/or

intracranial MRI were within normal limits, infectious disease testing was not required for inclusion and no standardisation of the treatment protocol was obtained. Medical management was also tailored to individual needs and therefore some dogs might have received additional medication, such as anti-epileptic drugs and mannitol. Additionally, dogs did receive different treatment protocols prior to admission and diagnosis. Results might have been biased towards more severely affected dogs by only including dogs with a CSF pleocytosis, dogs with abnormal intracranial imaging and dogs with clinical signs of raised ICP when CSF analysis was not performed. Definitive post mortem diagnosis was available in almost half of the dogs (14/30) that died within 1 week after diagnosis in the hospital. This might also implicate a bias towards the more severely affected cases, as dogs that die or are euthanized within a hospital environment are more likely to have post-mortem examination performed.

Conclusions

Twenty-six percent of dogs diagnosed with MUA in this study died within one week after diagnosis, emphasizing the need for evaluation of short-term prognostic factors. Presence of a decreased mentation at time of presentation, presence of seizures, and increased neutrophil percentage in the CSF were significantly associated with death within 7 days after diagnosis. The results of this study might be important when considering the overall prognosis of dogs with MUA and managing expectations of owners and hospital staff.

General Discussion and Conclusions

The main goal of this thesis was to gain more insight in the clinical presentation, diagnostic findings, treatment and prognosis of dogs diagnosed with MUA.

The typical clinical presentation of dogs with MUA is well described but a substantial number of large dogs have been included in multiple studies. Specific data regarding clinical presentation, diagnostic findings and long-term outcome are currently lacking about this group of dogs.

The same accounts for dogs diagnosed with MUA only affecting the spinal cord. As the disease is generally considered fatal if left untreated, multiple (prospective and retrospective) studies have been published, investigating different treatment protocols including one or multiple immunosuppressive drugs. These drugs however add a substantial cost to the treatment, which might be a limiting factor for some owners. Sole prednisolone therapy has generally been associated with poor outcome or very short (median) STs compared to combination therapy with other immunosuppressive agents. Two studies were conducted, a retrospective study to evaluate three historically used sole prednisolone treatment protocols, and a prospective study comparing one of the sole prednisolone treatment schedules with combination therapy with ciclosporine.

As stated above, MUA is considered a fatal disease, so multiple studies have been performed investigating potential prognostic factors for dogs with MUA. Although these studies evaluated a large amount of clinical and imaging variables, only few clinically useful factors have been identified so far. Additionally, as a lot of studies focus on long-term survival, little is currently known about initial response to treatment and short-term (one week) survival in dogs diagnosed with MUA.

Clinical presentation and diagnostic findings

Clinical presentation

In a first study (**Part I, Chapter 1**) we evaluated the differences in clinical presentation, diagnostic findings and long-term survival between small (<15kg) and large (>15kg) dogs diagnosed with MUA. The study included 111 dogs, including 28 (25%) large dogs and 83 (75%) small dogs. Large dogs were found to present significantly more often with a decreased mentation, but no other differences could be identified regarding clinical presentation, diagnostic findings and long-term survival between both groups.

MUA is generally considered a syndrome affecting small, toy and terrier breed dogs (Munana and Luttgen, 1998; Adamo et al., 2007; Talarico and Schatzberg, 2010; Coates and Jeffery, 2014). Interestingly, a quarter of dogs in the presented study were large dogs, so ignoring this population of dogs would underestimate the prevalence of MUA in the canine population. Overall, a total of 45 large dogs have been reported between 1998 and 2015 (Gregory et al., 1998; Munana and Luttgen, 1998; Cherubini et al., 2006; Fliegner et al., 2006; Zarfoss et al., 2006; Coates et al., 2007; Pakozdy et al., 2009; Smith et al., 2009; Wong et al., 2010; Flegel et al., 2011; Lowrie et al., 2013; Estey et al., 2014; Barnoon et al., 2015; Beckmann et al., 2015; Mercier and Barnes Heller 2015), with a similar breed distribution compared to our study. Adding the 28 dogs of our study gives a total number of 73 large dogs described. The Labrador Retriever (13/73; 17%), the English Springer Spaniel (9/73; 12%) and the Golden Retriever (7/73; 10%) were the three most commonly affected breeds over all studies. It is however not clear whether this reflects the popularity of those breeds in the respective countries or a specific breed predisposition for MUA. Therefore, MUA should be considered a differential diagnosis in dogs other than small or toy breeds that have signs suggestive of inflammatory CNS disease.

Although female predominance is a widely held belief in GME (Cordy, 1979; Russo, 1979; Braund, 1985; Bailey, 1986; Sorjonen, 1990; Munana and

Luttgen, 1998), no statistical difference in female:male ratio could be found in more recent studies (Talarico and Schatzberg, 2010; Granger et al., 2010). A total of 194 dogs was investigated in this thesis, including 101 males (52%) and 93 females (48%), revealing no difference in female:male ratio.

As stated in the introduction, dogs affected with NE were predominantly under 4 years old whereas the peak age for GME was 4-8 years (Granger et al., 2010). Historically, NME was described in Pug dogs with ages ranging from 6 months to 7 years (Cordy and Holliday, 1989), whilst dogs with a histopathological diagnosis of GME had ages ranging from 6 months to 12 years (Munana and Luttgen, 1998). In a series of 60 Pugs with NE (Levine et al., 2008), the median age was 18 months. In the presented studies in this thesis, median age at time of presentation was around 4 years in all studies (54 months (**Part I, Chapter 2**), 48 months (**Part II, Chapter 3**), 52.5 months (**Part III, Chapter 5**)). On the contrary, median age at time of presentation was 28 months in the prospective study (**Part II, Chapter 4**), which is remarkable younger. This can however be explained by the fact that all dogs in this study are breeds previously described to develop NE.

As 8% of dogs diagnosed with GME presented with neurological deficits suggestive of a myelopathy (Granger et al., 2010), we performed a second study (**Part I, Chapter 2**) evaluating the clinical presentation, diagnostic findings and long-term survival in 21 dogs diagnosed with SO-MUA. In our study, an acute or chronic onset of a lesion affecting the T3-L3 spinal cord segments resulting in ambulatory paraparesis was considered the most common clinical presentation. Thirty-three dogs with MUA only involving the spinal cord were previously reported, including one dog with paraplegia (Cherubini et al., 2006; Griffin et al., 2008; Wong et al., 2010), whereas no dog with paraplegia was included in our study. Unfortunately, more specific details were lacking for the majority of the previously published dogs. As the clinical presentation and diagnostic findings are not specific for SO-MUA, this disorder should be included in the differential diagnosis for all dogs presenting with different degrees of spinal cord dysfunction.

Pain on spinal palpation was present in 71% of dogs with SO-MUA in our second study, which is comparable to the 86% stated in a recent study (Cardy et al., 2015). Paraspinal hyperesthesia (Sorjonen, 1990) and cervical hyperesthesia (Cordy and Holliday, 1989) were previously described in GME and NME, respectively. As spinal pain reflects the involvement of the meninges, and/or vertebrae (vertebral periosteum), and/or nerve roots or spinal nerves (Da Costa, 2012), this cannot be considered a typical finding for SO-MUA. However, it is important for the clinician to understand that MUA should be in the differential diagnosis for dogs presenting with any form of spinal hyperesthesia.

Clinical diagnostic findings

Although presence of leucocytosis on CBC has previously been reported in dogs with GME (Thomas and Eger, 1989; Sorjonen, 1990; Tipold, 1995), nothing is currently known about prevalence and prognostic value. Throughout the different chapters, leucocytosis was present in approximately 15% of cases where CBC was available for review (13% for **Part I, Chapter 1** and **Part III, Chapter 5**; 9.5% for **Part I, Chapter 2**; 14% for **Part II, Chapter 3**; and 17% for **Part II, Chapter 4**). In **Part III, Chapter 5**, statistical analysis was performed to investigate whether presence of leucocytosis was associated with survival after 7 days, but this did not reach significance (P=0.103). In the other chapters, no further statistical analysis was performed.

MR imaging

MR imaging is considered the most sensitive imaging modality for detecting intracranial lesions, but up to 7% of scans showed no lesion on T2WI in dogs with MUA (Talarico and Schatzberg, 2010; Granger et al., 2010). Overall, the sensitivity of imaging in identifying all inflammatory lesions suspected from the neurological examination remained quite low (<60%) (Granger et al., 2010). In our first study (**Part I, Chapter 1**), the presence of intracranial lesions on MRI was part of the inclusion criteria, but no differences in MRI findings could be detected between small and large dogs.

Although studies looking into the use of MRI for differentiating between histopathologically confirmed cases of GME, NME or NLE are lacking, MRI findings in the different disease entities have been fairly well described previously (Cherubini et al., 2006; von Praun et al., 2006; Flegel et al., 2008; Young et al., 2009; Talarico and Schatzberg, 2010; Coates and Jeffery, 2014). On the contrary, MRI findings have been only described for 4/33 dogs reported with SO-MUA, revealing no abnormalities in 1 dog, and multifocal poorly demarcated T2W hyperintensities with variable contrast enhancement in 2 dogs (Cherubini et al., 2006; Wong et al., 2010). Therefore, we performed a second study (**Part I, Chapter 2**) to describe the specific MRI findings in 21 dogs with SO-MUA. No lesions were visible on sagittal T2WI or T1WI in 10% of cases, comparable to the 7% reported for MUA (Talarico and Schatzberg, 2010; Granger et al., 2010). All visible MRI lesions in our study were extensive, ill-defined, intramedullary, hyperintense on T2WI and isointense on T1WI. Parenchymal contrast enhancement was seen in 18 lesions (86%), and 17 lesions (81%) showed contrast enhancement of the overlying meninges.

Interestingly, although dogs were not allowed to have clinical signs or neurological examination results suggestive of intracranial involvement for inclusion in our second study (**Part I, Chapter 2**), additional MR images of the brain were included in the field of view of the cervical MRI in 2 dogs, showing additional lesions (suggestive of MUA) in both cases. As intracranial images were only available in 2 dogs, it is currently unclear (1) if these brain abnormalities represent a multifocal nature of the disease or cranial extension of the cervical inflammatory lesions, and (2) if inflammatory brain lesions are currently under diagnosed in dogs with SO-MUA and if SO-MUA could therefore be considered a more generalised inflammatory disease process, a meningoencephalomyelitis.

CSF analysis

Results on CSF analysis throughout the different chapters should be interpreted with caution as a CSF pleocytosis is considered an inclusion criterion for all studies. Overall, TNCC was normal in 2% of cases, including 3

dogs with histopathologically confirmed GME, NME or NLE in **Part III, Chapter 5**; and 1 dog without necropsy in **Part II, Chapter 4**. TP concentration was not set as an inclusion criterion in all studies, and TP measurements were available in 157/194 cases, revealing normal values in 36/157 cases (23%).

Although exclusion of regional infectious causes is a proposed diagnostic criterion for MUA (Granger et al., 2010), infectious disease testing was lacking in 52/187 cases (28%) in this thesis. All studies were performed in Belgium or the United Kingdom, but no prevalence studies for *T. gondii, N caninum* or CDV are available apart from two studies performed in the mid 90's in Belgium (Vanparijs et al., 1991; Barber et al., 1998). These studies revealed that approximately 10% of dogs in Belgium was seropositive for *N. caninum* (Barbet et al., 1998) and that no *T. gondii* oocysts were detected on examination of 2324 faecal samples of dogs and cats (Vanparijs et al., 1991). However, infectious disease testing should still be encouraged in all dogs suspected of MUA to exclude possible infectious agents as much as possible.

Treatment

Aggressive immunosuppressive therapy was initiated at time of diagnosis in all dogs included in all chapters of this thesis. This is important as early and aggressive therapy might improve survival in dogs with MUA (Barnoon et al., 2015). In the literature, immunosuppressive therapy is sometimes only initiated after results of infectious disease testing returned negative, complicating comparison between different study designs.

Treatment with sole prednisolone therapy

The exact aetiology and pathophysiology of MUA remain unknown, but as one or several different factors are believed to trigger an excessive immunologic response resulting in inflammatory changes in the CNS (Flegel et al., 2011), immunosuppressive therapy is considered the cornerstone of medical treatment. Treatment with glucocorticosteroids (mostly prednisolone)

only is generally associated with shorter survival times compared to combination therapy with other immunosuppressive agents (Munana and Luttgen, 1998; Jung et al., 2007; Pakozdy et al., 2009; Granger et al., 2010; Flegel et al., 2011; Mercier and Barnes Heller, 2015). Therefore, several treatment protocols using different immunomodulating drugs have been reported (Sisson et al. 1989; Gregory et al., 1998; Munana and Luttgen, 1998; Adamo and O'Brien, 2004; Gnirs, 2006; Zarfoss et al., 2006; Adamo et al., 2007; Coates et al., 2007; de Stefani et al., 2007; Feliu-Pascual et al., 2007; Uriarte et al., 2007; Jung et al., 2007; Menaut et al., 2008; Pakozdy et al., 2009; Smith et al., 2009; Granger et al., 2010; Kang et al., 2009; Wong et al., 2010; Flegel et al., 2011; Jung et al., 2012; Jung et al., 2013; Lowrie et al., 2013; Beckmann et al., 2015; Mercier and Barnes Heller, 2015; Barnoon et al., 2015; Lowrie et al., 2016).

Although combination therapy might result in a better long-term outcome in MUA, adding more expensive immunosuppressive therapies to the glucocorticoid protocol might be financially impossible in a clinical setting. Additionally, at the institution where our third study (**Part II, Chapter 3**) was conducted, dogs with MUA were historically only treated with prednisolone and the personal perception of the authors was that dogs were doing considerable well on this therapy. In the literature, initial prednisolone doses ranged from 0.34-30mg/kg/d (Munana and Luttgen, 1998; Jung et al., 2007; Pakozdy et al., 2009; Flegel et al., 2011; Mercier and Barnes Heller, 2015), but only one study (Talarico and Schatzberg, 2010) was providing a clinically useful treatment schedule. Therefore, the first aim of our third study was to retrospectively evaluate the clinical use of three different treatment schedules, and to provide a clinically useful sole prednisolone treatment schedule.

In this third study, we evaluated 38 dogs diagnosed with MUA that received oral prednisolone therapy in 3 different historically used tapering schedules (3, 8, and 18-week tapering prednisolone schedule). A significant difference was found between the three treatment schedules, with the highest number of deceased dogs in the 8-week treatment group (57%), followed by the 3 (26%) and 18-week (0%) treatment groups. It is possible that dogs receiving a longer and more immunosuppressive protocol might have a better outcome, but

this result should be confirmed in future studies. This 18-week treatment schedule is the same schedule as proposed by Talarico and Schatzberg (2010), so the authors would advise further clinical use of this schedule. However, as the initial dose is 3mg/kg/d, side effects might be significant.

Combination therapy with ciclosporine

Although ciclosporine was previously investigated as sole and add-on treatment for dogs diagnosed with MUA, no specific treatment protocols have currently been described (Adamo and O'Brien, 2004; Gnirs, 2006; Adamo et al., 2007; Jung et al., 2007; Pakozdy et al., 2009; Kang et al., 2009; Jung et al., 2012; Jung et al., 2013). Therefore, we performed a fourth study (**Part II, chapter IV**) to describe a clinically useful treatment protocol and to compare sole prednisolone therapy with combination therapy with ciclosporine in 12 dogs. The sole prednisolone protocol used in this fourth study was the same as the 18-week prednisolone schedule previously described in the third study. The described doses as add-on therapy for ciclosporine range from 6-30mg/kg/d (Adamo and O'Brien, 2004; Gnirs, 2006; Adamo et al., 2007; Jung et al., 2007; Pakozdy et al., 2009; Kang et al., 2009; Granger et al., 2010; Jung et al., 2012; Jung et al., 2013), but we decided to work with 5 mg/kg/d, as this dose is also known to cause some degree of immunosuppression (Archer et al., 2014). Although the difference in MST between both treatment groups seems clinically important (87 versus 567 days), no statistically significant results could be obtained. A power analysis was performed (non-parametric repeated sampling) revealing that 74 additional dogs were necessary in each treatment group to reach significantly different hazard ratios, which is quite a substantial number. Additionally, no previously established prognostic factors including clinical and diagnostic factors could be confirmed in this study.

Combination therapy with cytosine arabinoside

Investigation of the use and efficacy of cytosine arabinoside as an adjunctive therapy in canine MUA was not a primary goal of this thesis.

However, a total of 102 dogs treated with glucocorticosteroids plus cytosine arabinoside were recruited for the first (**Part I, Chapter 1**), second (**Part I, Chapter 2**) and fifth (**Part III, Chapter 5**) study. It should be noted that the same pool of dogs was used for the first and fifth study. Cytosine arabinoside can be administered either as a CRI (doses ranging from 100-300mg/m2 over 8-24 hours) or as 4 SC injections of 50mg/m^2 in 48 hours (200mg/m^2 in 48h) (Zarfoss et al., 2006; de Stefani et al., 2007; Menaut et al., 2008; Smith et al., 2009; Lowrie et al., 2013; Lowrie et al., 2016). Crook et al. (2013) showed that CRI administration of cytosine arabinoside provided a steady state concentration over the time it was administered compared to a rapid absorption and elimination when administered subcutaneously. A recent clinical study revealed a significantly better 3-month-survival in dogs initially receiving a CRI of cytosine arabinoside compared to the SC route (Lowrie et al., 2016).

In a fifth study (**Part III, Chapter 5**), we evaluated short-term outcome and prognostic factors in 116 dogs diagnosed with MUA. Eighty-eight dogs (85%) received additional cytosine arabinoside therapy; 69 via the subcutaneous and 19 via the intravenous route. Administration of cytosine arabinoside was significantly associated with a better outcome, but administration of a CRI was associated with a poor outcome compared to SC administration. Most likely, this is highly biased at the level of the clinician, whom would possible opt for IV cytosine arabinoside in more severely affected dogs. In the second study (**Part I, Chapter 2**), no significant difference in survival could be found for dogs receiving additional cytosine arabinoside therapy. As only 1/14 dogs (7%) were receiving a CRI of cytosine arabinoside, no conclusions can be drawn from this study regarding the route of administration.

Prognostic factors and outcome

Prognostic factors

As MUA is generally considered a fatal disease (Munana and Luttgen, 1998), multiple studies attempted to identify prognostic factors. Unfortunately,

different studies revealed conflicting results, making the majority of findings inapplicable in a clinical setting. Currently, the following established prognostic factors for dogs with MUA are available in the literature: 1) younger age at time of diagnosis was significantly associated with improved survival (Oliphant et al., 2016), 2) presence of seizures or altered mentation were significantly associated with shorter survival (Bateman and Parent, 1999; Coates et al., 2007; Granger et al., 2010); 3) presentation within 7 days of onset of clinical signs was significantly associated with longer survival (Barnoon et al., 2015); 4) abnormal serial CSF analysis was significantly associated with relapse and poor outcome (Lowrie et al., 2013); and 5) mass effect, loss of cerebral sulci and foramen magnum herniation on MRI were all significantly associated with death in dogs with MUA, however the clinical prognostic power was low for those findings and none of them was predictive of long-term outcome (Lowrie et al., 2013; Lowrie et al., 2016).

In our second study (**Part I, Chapter 2**), we investigated multiple possible prognostic factors in dogs with SO-MUA including clinical, diagnostic and treatment factors, but no association with long-term outcome could be made. On the contrary, our fifth study (**Part III, Chapter 5**), in agreement with previous studies, revealed that decreased mentation at presentation and presence of seizures were significantly associated with poor short-term outcome. Increased percentage of neutrophils on cerebrospinal fluid analysis also was significantly associated with poor short-term outcome, but no clinically useful cut-off values could be identified. None of the previously established prognostic factors could be confirmed in our fourth study (Part II, Chapter IV), although results should be interpreted with caution as only a limited number of dogs (n=12) was involved in this prospective randomized study.

Outcome

Although several studies have focused on long-term survival, little is known about short-term survival and initial response to therapy of dogs diagnosed with MUA. A recent study reported that 56% of dogs diagnosed with and treated for MUA died or were euthanized with a MST of 2 days (Lowrie et

al., 2013). In our fifth study (**Part III, Chapter 5**) we therefore evaluated the short-term survival in 114 dogs with MUA, revealing that 26% of dogs died within 7 days after diagnosis despite initiation of early and aggressive treatment. It seems therefore important to include this group of dogs when considering the overall prognosis of dogs with MUA. As more than 50% of the included dogs was alive after 7 days, no MST could be calculated in this study.

On the contrary, several studies using different treatment protocols resulting in different long-term survival times have been reported, with resulting MST ranging from 2 – 1834 days (Sisson et al. 1989; Gregory et al., 1998; Munana and Luttgen, 1998; Adamo and O'Brien, 2004; Gnirs, 2006; Zarfoss et al., 2006; Adamo et al., 2007; Coates et al., 2007; de Stefani et al., 2007; Feliu-Pascual et al., 2007; Uriarte et al., 2007; Jung et al., 2007; Menaut et al., 2008; Pakozdy et al., 2009; Smith et al., 2009; Granger et al., 2010; Kang et al., 2009; Wong et al., 2010; Flegel et al., 2011; Jung et al., 2012; Jung et al., 2013; Lowrie et al., 2013; Beckmann et al., 2015; Mercier and Barnes Heller, 2015; Barnoon et al., 2015; Lowrie et al., 2016). Our first study (**Part I, Chapter 1**) revealed an overall MST of 272 days. When separately evaluated for large and small dogs, the MST was 106 and 281 days, respectively, which is all-comparable to the published literature for MUA. As no specific survival data were available for dogs with SO-MUA, we performed a second study (**Part I, Chapter 2**) specifically evaluating the long-term outcome in those dogs. The study revealed a MST of 669 days, and 48% of dogs had died or been euthanized because of the disease.

In our third study (**Part II, Chapter 3**) we specifically evaluated the long-term survival in dogs with MUA receiving three different treatment protocols of sole prednisolone therapy. For this therapy, reported MSTs were 28 – 357 days (Granger et al., 2010), 91 - 329 days (Flegel et al., 2011) and 602 days (Mercier and Barnes Heller, 2015). As more than 50% of the dogs was alive or censored for outcome calculations at time of data capture in our study, no overall MST could be calculated. However, the MST was 180 days in the 8-week treatment group, which appears to be the group with the highest percentage of deceased and relapsed dogs. Our fourth study (**Part II, Chapter IV**) revealed an overall MST of 115 days in a prospective study including 12

dogs with MUA that were treated with sole prednisolone or combination therapy of prednisolone with ciclosporine, although no difference in MST could be demonstrated between both treatment groups.

Overall, long-term outcome data are available for 182 dogs in this thesis, revealing that 98/138 dogs (71%) died because of MUA, which is higher compared to the previously reported 56% (Lowrie et al., 2013). It has been previously mentioned that most dogs with MUA or GME that die, do so within the first three months after diagnosis (Thomas and Eger, 1989; Smith et al., 2009; Lowrie et al., 2013). For the presented studies, 63/98 dogs (65%) died within 3 months after diagnosis. In both prospective studies (**Part II, Chapter 3 and 4**) and the retrospective study regarding SO-MUA (**Part I, Chapter 2**), 7/34 dogs (21%) died because of MUA within 7 days after diagnosis, which is comparable to the results of the fifth study (**Part III, Chapter 5**), where 26% of dogs with MUA died within one week after diagnosis.

Limitations

MUA is a clinical diagnosis that is based on a combination of signalment, neurological examination results and cross-sectional imaging findings in the absence of histopathological confirmation (Munana and Luttgen, 1998; Adamo et al., 2007; Talarico and Schatzberg, 2010; Coates and Jeffery, 2014). Specific diagnostic criteria are currently available, but this might bias studies towards more severely affected cases. To illustrate this, dogs with normal MRI findings (reported in 7-10% of dogs with MUA) or normal CSF analysis (reported in 3-57% of dogs with MUA) do not fit those inclusion criteria and will mostly not be included in prospective or retrospective studies, which also accounts for all studies in this thesis. Additionally, dogs were included in **Part II, Chapter 3** based on CT imaging whereas MRI is generally considered the diagnostic imaging modality of choice for diagnosis of MUA. Four out of 5 reported studies in this thesis are retrospective studies. These carry the pitfall that they only carry limited standardization of patient assessment (different people and institutions involved) and therapy (different institutions use different treatment protocols and have different follow-up practices). Additionally, there

might be debate about which infectious disease testing to perform or not to perform in dogs diagnosed with MUA. PCR testing is expensive for the pet owner and might therefore be declined, and carries the risk for false negative results. On the contrary, as long as histopathological confirmation is lacking, no certainty can be obtained about the underlying pathology (GME, NME or NLE) and so conclusions should be drawn with caution.

Future perspectives

Large, prospective, multi-centre studies are needed to further investigate the findings of this thesis. The first study revealed that 25% of dogs affected with MUA are large dogs (>15kg), but it remains currently unclear whether this reflects a different disease entity, or just the same disease that is emerging in the whole canine population. A study to specifically look into clinical, clinicopathological, imaging and histopathological findings in large dogs with MUA is needed. The same accounts for dogs with SO-MUA, as currently only a small cohort of 21 dogs and the associated MRI findings were described. On-going research investigating clinical presentation, diagnosis and outcome in those dogs is warranted.

Although the criterion-referenced standard for a clinical trial is a randomized, placebo-controlled, double-blinded, prospective study, it is generally accepted that use of a placebo control treatment group is unethical because dogs with MUA have a poor outcome without treatment. Large, multi-centre studies are necessary to compare different treatment products and schedules in a larger patient population.

Together with the investigation of additional clinical and outcome data, it is also important that the clinical diagnosis of MUA becomes more and more certain based on several inclusion criteria. As more and more institutions currently have high-field MRI available, evaluation of more criteria to differentiate between NME, NLE and GME can possibly aid the clinician in making a presumptive diagnosis. At the same time, authors should be motivated to use the same inclusion criteria to facilitate reliable comparison between studies.

Conclusions

In conclusion, this thesis revealed novel information regarding clinical presentation, diagnosis, treatment and prognosis for dogs with MUA. Different breeds and different regions of the central nervous system can be affected by MUA. Typical MRI findings in dogs with MUA only affecting the spinal cord were provided, but further investigations regarding dogs with paraplegia and dogs with concurrent intracranial involvement are warranted. Overall, the prognosis of MUA is guarded. Twenty-six percent of dogs will die or be euthanized within one week after diagnosis despite treatment, and a large number of dogs will die or be euthanized because of MUA on the long-term. Further studies are warranted to provide more accurate and clinically useful diagnostic variables and prognostic factors. Large, prospective, randomized multi-centre studies should be performed to investigate different immunomodulating treatment protocols.

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Summary

Meningoencephalomyelitis of unknown aetiology (MUA) in dogs is a complex and incompletely understood immune mediated CNS disorder. There is controversy and uncertainty about several aspects of the clinical presentation, diagnosis, treatment and prognosis of the different non-infectious inflammatory central nervous diseases this disorder covers. In this PhD thesis, several aspects concerning the clinical presentation, diagnostic findings, treatment, prognosis and outcome were evaluated.

As a **general introduction,** a brief overview is given about the current literature on the clinical presentation, diagnosis, treatment, outcome and prognosis of MUA in dogs. Additionally, points of controversy and suggestions for further research are mentioned.

Part I describes the clinical presentation and diagnostic findings in specific groups of dogs with MUA. In **Chapter 1**, the clinical presentation, diagnostic findings and long-term outcome of 28 large dogs (>15kg) diagnosed with MUA were assessed. Our study showed that 25% of dogs diagnosed with MUA were considered large dogs (28/111 dogs). They significantly more often presented with decreased mentation compared to their small counterparts. Age, gender, duration of clinical signs prior to diagnosis, presence of seizures or cluster seizures, variables on complete blood count and cerebrospinal fluid analysis, and all variables on MRI were not significantly different between small and large dogs. The MST was 281 and 106 days for the large and small dogs, respectively, but no significant difference in survival curves could be detected. In **Chapter 2**, the clinical presentation, MRI findings and long-term outcome in 21 dogs with MUA only affecting the spinal cord were evaluated. These dogs mainly presented with an acute (43%) or chronic (52%) onset ambulatory paraparesis (67%) affecting the T3-L3 spinal cord segments, with presence of spinal hyperesthesia in 71% of cases. MRI revealed no abnormalities in 10% of dogs, and revealed an ill-defined, intramedullary lesion that was hyperintense on T2WI and isointense on T1WI with presence of parenchymal and/or meningeal enhancement in 86% and 81% of cases, respectively. Regarding outcome, 48% of dogs died or was euthanized because of the disease, with an overall median survival time of 669 days.

Part II describes two possible treatment options for dogs with MUA. In **Chapter 3**, we retrospectively assessed three different sole prednisolone treatment schedules (3, 8 and 18-weeks tapering schedule) in 38 dogs with MUA. Overall, 37% of dogs died or was euthanized because of MUA, and a significant difference in ST was seen between the three treatment schedules. Surprisingly, the highest number of dogs that died because of MUA was seen in the 8-week treatment schedule (56%), followed by the 3-week (26%) and 18-week (0%) treatment schedule. As no dog had deceased in the group receiving the most prolonged immunosuppressive schedule (18-weeks tapering schedule), it was suggested that dogs might potentially have a better outcome using this schedule.

Chapter 4 prospectively evaluated sole prednisolone therapy and combination therapy with ciclosporine in 12 dogs diagnosed with MUA. Two clinically useful treatment protocols were described, including the previously described 18-week tapering prednisolone treatment protocol and combination therapy of the same schedule with 5mg/kg/d ciclosporine. The MST was 87 and 567.5 days for the sole prednisolone and combination therapy groups, respectively, but no difference in survival curves could be detected. None of the previously established prognostic factors including clinical and diagnostic factors could be confirmed.

Part III, Chapter 5 evaluated prognostic factors for one-week or short-term survival in 116 dogs diagnosed with MUA. Overall, 26% of dogs died within 7 days after diagnosis despite initiation of early and aggressive immunosuppressive therapy. Decreased mentation at presentation, presence of seizures and increased percentage of neutrophils on cerebrospinal fluid analysis were significantly associated with a poor 7-day survival. Unfortunately, no clinically useful cut-off value could be identified for the increased percentage of neutrophils, limiting the clinically usefulness of this variable.

In **conclusion**, the studies presented in this thesis proved new information considering clinical presentation, diagnosis, treatment, outcome and prognosis in dogs diagnosed with MUA, but further studies are warranted to confirm those findings.

Samenvatting

Meningoencefalomyelitis van onbekende etiologie (MOE) bij de hond is een complexe aandoening van het centrale zenuwstelsel. Verschillende aspecten van de klinische presentatie, de diagnostische criteria, de behandeling en de prognose zijn onderwerp van discussie. In deze doctoraatsthesis hebben we verschillende aspecten van de klinische presentatie, de diagnostische bevindingen, de behandelingsmogelijkheden, en de lange en korte termijn prognose bestudeerd.

In de **algemene introductie** werd een kort literatuur overzicht gegeven van de huidige details over de klinische presentatie, de diagnostische bevindingen, de verschillende behandelingsmogelijkheden en de prognose van honden met MOE. Bijkomend zijn ook enkele punten van discussie besproken en zijn er mogelijke toekomstige onderzoeksmogelijkheden aangehaald.

Deel I van deze doctoraatsthesis beschrijft de klinische presentatie en de diagnostische bevindingen bij twee specifieke groepen binnen MOE. **Hoofdstuk 1** bespreekt de klinische presentatie en de diagnostische bevindingen, gecombineerd met de lange-termijn prognose bij 28 grote honden (> 15kg). Onze studie toonde aan dat 25% van de honden die gediagnosticeerd werden met MOE tot deze groep van grote honden behoorden. Bovendien werden zij significant vaker aangeboden met klachten van verminderd bewustzijn vergeleken met kleine honden (< 15kg). Leeftijd, geslacht, duur van de klinische symptomen voor de diagnose gesteld werd, aanwezigheid van epileptische aanvallen of cluster epilepsie op het moment van de diagnose, variabelen op het algemene bloedonderzoek en op onderzoek van het hersenvocht, en alle parameters die werden onderzocht op de MRI beelden, waren niet verschillend tussen grote en kleine honden. De mediane overlevingstijd van de grote honden was 281 dagen, en deze van de kleine honden was 106 dagen. Tussen beide overlevingstijden kon er geen significant verschil worden vastgesteld.

Hoofdstuk 2 bespreekt de klinische presentatie, de MRI bevindingen en de prognose van 21 honden met MOE die zich enkel presenteren met klachten komende van het ruggenmerg. Deze honden werden meestal aangeboden met klachten van een acute (43%) of chronische (52%)

ambulatoire paraparese (67%). Meestal werd het letsel gelokaliseerd ter hoogte van ruggenmergsegmenten T3-L3, en bij 71% van de honden werd er pijn gevonden bij palpatie van de wervelkolom. Een MRI scan van de wervelkolom toonde geen afwijkingen bij 10% van de honden. Indien er wel een letsel gevisualiseerd werd, dan was dit meestal slecht omschreven, intramedullair, hyperintens op T2-gewogen beelden en isointens op T1 gewogen beelden. Parenchymale of meningeale contrastcaptatie was aanwezig in respectievelijk 86% en 81% van de gevallen. Achtenveertig procent van de honden stierf of werd geëuthanaseerd ten gevolge van de aandoening, en dit met een mediane overlevingstijd van 669 dagen.

Deel II van de doctoraatsthesis beschrijft twee behandelingsmogelijkheden bij honden met MOE. **Hoofdstuk 3** evalueert retrospectief een behandeling met monotherapie prednisolone bij 38 honden met MOE. Hierbij werden 3 verschillende behandelingsschema's met prednisolone met elkaar vergeleken, namelijk een 3-weken, een 8-weken en een 18-weken afbouwend prednisolone schema. Er werd een significant verschil in overleving gevonden tussen deze drie behandelingsschema's. De meeste honden stierven in de groep die behandeld werd met het 8-weken schema (56%), gevolgd door het 3-weken (26%) en het 18-weken (0%) schema. Gezien er nog geen honden gestorven waren in de groep die het 18-weken schema, het meest immunosuppressieve schema, toegediend kregen, werd gesuggereerd dat honden met MOE mogelijks een betere overleving hebben indien ze een meer immunosuppressief behandelingsschema krijgen.

Volgend op voorgaande studie, werd een prospectieve studie uitgevoerd die beschreven staat in **hoofdstuk 4**. In deze studie werd het verschil in overleving bekeken bij honden met MOE die twee verschillende behandelingsschema's kregen. Een eerste groep honden werd enkel behandeld met een schema identiek aan het in het vorige hoofdstuk beschreven 18-weken schema prednisolone, en bij een tweede groep honden werd deze behandeling gecombineerd met ciclosporine. De mediane overlevingstijd bedroeg respectievelijk 87 en 567.5 dagen voor de honden enkel behandeld met prednisolone en de honden behandeld met de combinatie prednisolone en ciclosporine. Deze mediane overlevingstijden waren niet significant

verschillend. Bovendien werden enkele vooraf beschreven prognostische factoren geëvalueerd, maar geen enkele factor kon in deze studie bevestigd worden.

Deel III van deze thesis evalueert de prognostische factoren voor de kans op overleving binnen de eerste week na diagnose van MOE. De resultaten van deze studie staan beschreven in **hoofdstuk 5**. Een totaal van 116 honden werd retrospectief geïncludeerd in deze studie, waarbij 26% stierf binnen de 7 dagen na diagnose ondanks er een vroegtijdige en agressieve behandeling werd ingesteld. Verminderd bewustzijn, aanwezigheid van epileptische aanvallen en de aanwezigheid van een verhoogd percentage neutrofielen op onderzoek van cerebrospinaal vocht op moment van diagnose, waren significant geassocieerd met een slechte kans op het overleven van deze eerste 7 dagen na diagnose. Jammer genoeg kon er voor het percentage neutrofielen geen cut-of waarde aangeduid worden, en dus is deze factor klinisch niet bruikbaar.

Als conclusie van deze thesis kan er gesteld worden dat er nieuwe informatie werd aangebracht inzake de klinische presentatie, de diagnostische criteria, de behandelingsmogelijkheden en de prognose van honden met MOE, maar verdere studies zijn nodig om onze bevindingen te bevestigen.

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Curriculum Vitae

Ine Cornelis werd geboren op 15 april 1984 te Antwerpen.

Zij behaalde in 2008 het diploma van master in de diergeneeskundige wetenschappen aan de Universiteit Gent. Aansluitend deed zij een rotating internship aan de Faculteit Diergeneeskunde van de Universiteit Gent, meteen gevolgd door een specialisatie opleiding in de neurologie (residency). Deze werd met succes afgerond in 2014 met het behalen van het Europese specialisten diploma (Diplomate of the European College of Veterinary Neurology).

Sinds april 2010 werkt zij als assistent neurologie aan de Vakgroep Kleine Huisdieren waar zij klinisch werk combineert met het afwerken van haar doctoraatsonderzoek. In 2014-2015 onderbrak ze haar ambt om een jaar te werken als Staff Clinician in Neurology and Neurosurgery aan de Royal Veterinary College in Londen.

Ine Cornelis is auteur of mede-auteur van verschillende wetenschappelijke publicaties. Zij was spreker op twee symposia en nam actief deel aan meerdere nationale en internationale congressen.

Dankwoord

Voilà, eindelijk is het zover, de openbare verdediging van mijn doctoraat! Hopelijk hebben jullie ervan genoten en zit je nu niet stiekem dit dankwoord te lezen terwijl ik vooraan sta te zweten. Indien dit wel het geval zou zijn: geniet ervan!

Beste promotoren, graag zou jullie toch allemaal even willen bedanken, want zonder jullie hulp had dit meesterwerk er nu niet gelegen.

Sofie, ik denk dat ik weet waar een deel van je grijze haar vandaan komt! Wat begon als een idee over stamcelonderzoek enkele jaren geleden, is uitgedraaid op een onderzoek over meningoencefalitis. Ik kan me het moment van die beslissing niet meer herinneren, maar het is toch een goede gebleken. Bedankt om van in het begin van dit verhaal in mij en mijn onderzoek te blijven geloven. Het eerste artikel heeft letterlijk bloed, zweet en tranen gekost, maar vanaf dan is het toch beter beginnen gaan. Bedankt om een luisterend oor te zijn, voor de gedetailleerde correcties en je ongezouten – maar daarom niet minder geapprecieerde - mening. Ik wens je alle succes toe met het Epicentrum project, en ik ben fier om er deel van te mogen uitmaken!

Luc, jouw liefde voor de neurologie werkt inspirerend! Na de masterproef en mijn internwerkje met case report hebben we het wat grootser aangepakt en dat resultaat ligt er nu. Bedankt om me te steunen in mijn onderzoek, mijn residency, mijn jaar naar het buitenland, … Ik mocht u tijdens mijn wachten dag en nacht opbellen, en ik stond er dan vaak van versteld hoe u met één blik op een myelogram voldoende had om de lokalisatie te bepalen! Die tijden liggen al eventjes achter ons, de myelogram avonturen zijn geschiedenis geworden, maar de fijne samenwerking is wel gebleven! Heel erg bedankt!

Ingrid, dankzij u konden we de prospectieve studie doen, en de mensen toch overtuigen van het nut om de hersenen van hun hond opnieuw te laten scannen. Ook al hebben we geen grote aantallen patiënten verzameld (maar niet getreurd, volgens de power analyse moeten we er nog maar 148 extra doen!), het heeft toch maar een mooi resultaat opgeleverd! Een dikke merci om alles altijd zo snel na te lezen en voor de bemoedigende mailtjes. Ook voor de hondjes zal je me in de toekomst nog wel eens zien verschijnen!

Steven, mijn vierde promotor. Vreemd genoeg kreeg ik mijn manuscripten meestal terug in een andere kleur dan waarin ik ze opgesteld had. Ook al zag ik dan soms de teleurstelling in je ogen, tot hiertoe is het uiteindelijk altijd goed afgelopen! Ik weet nog steeds niet waar je de ideeën voor studies vandaan blijft halen, misschien moet ik toch ook overschakelen van Fanta op koffie, en op een meer sportieve levenswijze/vakanties? Bedankt voor je constructieve manier van samenwerken en voor het blijvende geloof in mijn onderzoek. Ik vind het bijzonder jammer dat we elkaar door de afstand minder zien, maar we zullen zeker contact proberen te houden in de toekomst! Zorg goed voor Laura, kleine Lou en zijn broertje binnenkort, voor neefje Herman en voor hooghartige Flo.

Beste leden van de lees – en examencommissie, **Prof. Dr Kurt Houf**, **Prof. Dr. Saunders**, **Prof. Dr. Paepe**, **Dr. Devriendt**, **Dr. Van Soens**, **Prof. Dr. Poncelet**, bedankt om enkele uren (hopelijk geen dagen) van jullie leven te spenderen aan het nalezen van deze thesis. Ik heb zeer veel bijgeleerd van jullie vragen en bemerkingen, en dit werk zou dan ook zo mooi niet zijn zonder jullie hulp!

Mijn werk op de neurologie zou er natuurlijk ook niet zijn zonder mijn collega's! **Valentine**, bedankt om altijd een luisterend oor te zijn bij eventuele baby vragen of problemen! Bedankt voor jou aandeel als supervisor tijdens mijn residency! **Kimberley**, je gedrevenheid in het opzoeken van artikels en informatie werkt aanstekelijk. Ik heb er dan ook alle vertrouwen in dat je deze opleiding tot een goed einde zal brengen! **Marios**, as the newest member of the team, I can imagine life is sometimes hard sharing a desk with 3 women. Certainly when one of those women decides to change outfits in the office, poor you. If we can measure your motivation by the amount of eggs you are eating a day, you're doing a great job! **Dr. Tshamala**, binnenkort kan u eindelijk van een welverdiend pensioen genieten! Bedankt voor het geduld in de operatiezaal en om altijd bereid te zijn een leven te redden in het weekend. **Daniela**, bedankt om mijn mede-resident te zijn. Ik ben blij dat je nu je American dream beleeft, heel veel succes met alles wat je doet! **Kenny**, bedankt om voor me in te springen tijdens mijn moederschapsrust en borstvoedingsverlof! Zorg goed voor Alix en Leon, en dat laatste deel van het examen komt zeker en vast ook nog

wel in orde! **Emilie**, jou schrijf ik natuurlijk aan met een klein beetje weemoed en een tikkeltje jaloezie. Fantastisch dat ook jij de sprong waagde om in Engeland te gaan werken. Eindelijk kan je ook naar Supervet kijken! Bedankt om toch een groot deel van de residency samen door te brengen, helaas zal ik je niet opvolgen als best geklede neurologie collega. **Iris**, merci natuurlijk nogmaals als lid van de lees – en examencommissie, maar toch ook om er te zijn in Bologna. Het was ongelooflijk aangenaam om er een bekend gezicht in de buurt te hebben! Nog heel veel succes bij Orion! **An**, last but not least, we zien elkaar ook niet veel meer, maar het is toch altijd nog gezellig! Gelukkig houdt Antita de “oude” bureau ook nog een beetje bij elkaar!

Ook mijn andere collega’s in Gent: bedankt voor de fijne samenwerking! Jullie allemaal persoonlijk aanspreken zou wat lang duren, maar er zijn toch enkele mensen die ik hier graag even persoonlijk zou willen bedanken (hopelijk vergeet ik dan niemand!). **Dominique**, mocht ik hersenen kunnen kopen zoals de jouwe, dan zou ik het zeker doen! Misschien moet ik overwegen om naast de switch van Fanta naar koffie en een sportievere levensstijl, ook de switch naar Nutella te maken? Bedankt voor de fijne samenwerking, en heel veel succes in je nieuwe functie! **Pascal**, nu we buiten onze studie ook nog iets anders gemeen hebben, kan het soms al eens deugd doen om je hart te luchten over slechte nachten, snotterende neuzen of lopende oren. Ook voor u het allerbeste in je nieuwe functie! **Pieter** en **Steven**, als we zo doorgaan zouden we nog wel eens een intern jaar kunnen worden met een record aantal diplomates! **Virginie**, bedankt voor de fijne babbels en het lunchen af en toe. Ik zal er als Dr. op toezien om me toch iets volwassener te kleden. **Lise V**, eindelijk heb ik terug iemand gevonden die de liefde en passie voor de hondjes deelt. Hopelijk kunnen je oren mijn gezaag nog een tijdje verdragen. Heel veel succes ook met je onderzoek! **Kaatje**, bedankt voor je altijd blijvende kritische interpretatie van de beelden. Ook hier heb ik misschien enkele grijze haren extra veroorzaakt (hopelijk zijn ze niet allemaal van mij!), maar weet dat ik je samenwerking en kennis enorm apprecieer! **Ingeborgh**, bedankt voor je onbeperkte inzet voor de honden, en voor het vertrouwen dat je hiervoor in mij stelt. Hopelijk kunnen ook wij onze samenwerking nog een hele tijd verderzetten! **Evelien**, ook al zien we elkaar minder, en is afspreken telkens

opnieuw een agendagewijze uitdaging, toch geniet ik altijd van de babbels! Ook jij staat voor een nieuw hoofdstuk in je leven, geniet er ten volle van! **Bieke**, wat ben ik blij dat jij bij de hondjes terechtgekomen bent! Het is een schip op zich, en dat varende houden vereist soms heel wat vaar – en roeikunsten, maar tot hiertoe lukt dat altijd wonderwel! Bedankt om naast een goede mama voor al die hartendiefjes ook een luisterend oor en superfijne collega te zijn! Geniet nu maar van de tijd met de kleine baby (de naam is tot het moment van dit schrijven nog altijd een verrassing) en dan beginnen we er nadien samen terug vol goede moed aan!

Almost 2 years ago now, I was working for one year as a neurologist at the Queen Mother Hospital for Animals in London. **Holger**, thank you so much for this year as part of your amazing team, and for the support and (statistical) help throughout the different studies. Although I always believed only women can multitask, you showed me that it is possible for some men as well! **Elsa**, I still miss the morning coffees (or hot chocolates) and the lovely chats. I can only wish you a lovely future with lots of diving holidays! I don't have Instagram, but I would otherwise be one of your "followers" for those amazing pictures! Miss you loads!! **Patrick**, **Stieven**, **Joe**, **Tom**, **Fran**, **Abbe**, **Alex** and **Danielle**, thank you all for letting me being part of the neuro – team! Keep up the good job, you are all amazing people! Hope we can meet soon! **Birgit**, life can be hard and very unfair, but I admire your strength and optimism! I love the Skype and WhatsApp messages from you, including the beautiful pictures (that don't make me feel jealous at all)! What a powerful women you are! I'm really looking forward to seeing you in a couple of days!

Nele en **Annelies**, ook wij zien elkaar wat minder, maar het stukje taart laatst heeft me toch ongelooflijk gesmaakt (en smaakte misschien wel naar meer?). Veel succes met jullie praktijk, en alle bewondering voor jullie harde werk daar! **Gosia**, wat een ongelooflijk sterke vrouw ben jij! Drie kindjes (ik herinner me nog altijd het moment dat we het sms'je kregen in de aula dat Simon geboren was – voor ons toen echt een "ver van mijn bed" show!) en ook bijna je doctoraat. Hopelijk tot binnenkort en veel succes op je nieuwe functie. **Jody**, de knapste van ons interngroepje, ook voor jou veel bewondering dat je

het leven als mama van Terence en Eloise kan combineren met je eigen praktijk. Eten we snel nog eens een broodje samen?

Koen en Tania, **Mark en Lore**, **Hans en Leen**, jullie hebben me er gratis en voor niets bijgekregen! Binnenkort zal de vriendengroep uit Leuven zowel bestaan uit tieners (pubers?), peuters en baby'tjes, we kijken er al naar uit!

Antita zonder Tony, bedankt voor de fijne babbels en de miljoenen kopjes thee en koekjes. Voor de zaterdagse en zondagse bezoekjes aan bestaande en onbestaande tweedehands beurzen. Voor de wandelingen met de honden en de hulp met dit doctoraat. Antita met **Tony** voor de discussies over bioproducten, dierenwelzijn en politiek. Ik sta nog altijd versteld van je enthousiasme soms! En natuurlijk ook een dikke zoen voor kleine Ernest en een knuffel voor Caillou (ik stel me nu zijn enthousiaste onthaalgeluiden voor).

Josine. We hebben samen al heel wat meegemaakt, maar onze vriendschap is er alleen maar sterker door geworden, en daar ben ik je oneindig dankbaar voor. Ook bij jou heb ik al liters thee gedronken en massa's koekjes gegeten. Het blijft leuk om met je van gedachten te wisselen over onze vele gemeenschappelijke interesses (de tuin, je vele dieren, interieurkeuzes, …). Bedankt om altijd een luisterend oor te zijn, en hopelijk kan ik dat ook voor jou nog heel lang blijven! **Dimitri**, ook jij heel erg bedankt om er al die jaren geweest te zijn!

Tim en **Bram**. Volgens mij kennen jullie als enige mijn aanleg voor zang en dans, ook al is het repertoire redelijk beperkt. Tim, bedankt voor je jarenlange hulp bij de hondjes, en de fijne wandelingen nadien. Het pack van toen is behoorlijk geslonken, zorg dus nog maar goed voor het laatste gemenerikje. Bedankt voor de vele telefoontjes, en om mijn frustraties en gezaag te blijven verdragen. Bram, wat zouden de jetsers (en tezamen hebben wij veeeel jetsers) zijn zonder jouw kennis over films, muziek, politiek (of wist jij wel wie de president van Oekraïne is?), … Tijdens de zomer – als we nog eens gaan zwemmen in de Ster – wil ik ook graag nog eens je beroemde paella paella eten!

Nele en **Jurgen.** Nele, bedankt om ondertussen al dik 25 jaar deel uit te maken van mijn leven! Om die steunpilaar te zijn in moeilijke en gemakkelijke momenten, voor een lach en een traan. Je bent een fantastische mama voor jullie drie kindjes, waaronder mijn metekindje Jules! Hopelijk kunnen we elkaar in de toekomst terug wat vaker zien, en kunnen we nog vele diner-dates houden! Bedankt dat ik doorheen de jaren bij jullie altijd welkom ben geweest!

Ludo en Tineke, **Wim en Leen**, **Ann en Stef**, bedankt om zo een fijne schone familie voor mij te zijn! Ook al wonen jullie dan aan de verkeerde kant van het water, het is fijn te weten dat we bij jullie altijd welkom zijn! Cleo en Lise, jullie worden binnenkort grote zussen van Wout zijn neefje, en ik beloof jullie dat jongetjes soms ook heel lief kunnen zijn.

Bomma's en **bompa**, dat ik nog 3 van mijn 4 grootouders heb, is bijzonder. Mijn peter zou op een dag als vandaag zeker en vast heel trots geweest zijn! We zien elkaar op dit moment niet zo vaak, maar ik koester wel heel fijne herinneringen aan de vele leuke momenten met jullie!

Mama en **papa**, ook al weet ik nog altijd niet heel goed vanwaar (of van wie) ik mijn liefde voor dieren heb, jullie hebben me altijd gesteund in mijn droom om dierenarts te worden! Mama, bedankt om er altijd voor ons te zijn, en voor de fantastische zorgen! Papa, ook wel daddy perfect (of Daddy P voor de vrienden), bedankt om mij gedurende verschillende jaren altijd naar mijn kot te brengen op zondag avond (na het nieuws!). Merci dat we altijd welkom zijn, en om zo'n toegewijde bomma en bompa te zijn voor Wout, Maarten en Jolien! **Bart en Annemie**, **Hans en Dymphna**, **Erwin**, en **Gerd en Lore**, wat zou mijn leven saai zijn zonder jullie (en stil). Gelukkig zien we elkaar nog altijd regelmatig aan de brug van den Azijn. Weet dat jullie altijd welkom zijn in Melle!

Rik, onze liefde is begonnen met Bob's verlies van zijn staart. Hij is er ondertussen niet meer, maar gelukkig is de liefde wel gebleven (mooi geformuleerd he!). Ondertussen mag ik je al "husband" noemen en is er zelfs een klein rondkruipend hummeltje bijgekomen. Bovendien weet ik nu ook dat Freddy Mercury blijkbaar geen deel uitmaakt van de Beatles … Bedankt om er altijd voor mij te zijn, om mijn grillen te verdragen (soms toch) en om zo'n fantastische papa voor Woutje te zijn! Lieve kleine **Wout**, mama ziet je zo super

graag! Ook al zou ik 's nachts soms graag willen dat je een "uit" knopje had, als je dan naar ons kijkt, lacht en een keer dada doet, dan zijn we het onmiddellijk weer vergeten! Hopelijk kan je opgroeien tot een fijn, zelfzeker en gelukkig mannetje!

Naast de mensen zijn er natuurlijk ook al wat diertjes de revue gepasseerd ondertussen. In het verleden enkele poezen in Merksem (Scaramouche, Antraciet, Sloeber, Vlekje, Minoes, …) maar toch was ik super gelukkig toen ik 7 jaar geleden mijn eerste hondje uit het asiel ging halen! Lieve **Sali**, wat heb je soms mijn leven (en interieur) tot een hel gemaakt, maar wat ik je altijd ongelooflijk graag gezien! Er komen nog altijd traantjes als ik aan je denk, en aan de vreselijke manier waarop ik je verloren ben. Het was aandoenlijk om je samen met **Wiebe** te zien, bij hem had je eindelijk je rust gevonden. Jullie zijn nu voor altijd terug samen, en daar kan ik alleen maar dankbaar en blij om zijn! Jullie schitteren op de cover van dit doctoraat!! **Lizzy**, een rode cocker, wat een beslissing! Je hebt wat speciale aandacht nodig, maar je past perfect in ons gezin! Hopelijk kunnen we van jou nog lange tijd genieten!

Last but not least, zijn er nog enkele hondjes die een belangrijk deel uitmaken van mijn leven, en waar ik me ook al gedurende verschillende jaren tezamen met verschillende mensen voor inzet. Sommigen onder hen zijn ondertussen prachtige sterretjes aan de hemel, andere zijn nog bij ons of genieten van hun meer dan welverdiende pensioen in een eigen mandje! Eén voor één zijn jullie bijzonder, met jullie eigen persoontje en karakter. Ik zal jullie alvast nooit vergeten!

Lieve Margriet, Lotte, Lovely, Simply, Yoda, Yuba, Schnappi, Kitana, Molly, Vita, Flappie, Softie, Madelief, Lelie, Bloesem, Margriet, Roos, Orchidee, Violet, Iris, Narcis, Jos, Jaak, Jenny, Jeanine, Leo, Ludo, Mindy, Toby, Tibo, Kwibi, Zohra, Mindy, Tommy, Sammy, Anglo, Norman, Crimson, Blue, Curly, Clinton, Backford, Matchbox, Marshall, Marcus, Kaydee, Bert, Ernie, Bonnie, Clyde, Tristan, Isolde, Romeo, Julia, Balou, Borre, Cyriel, Liesje, Sofietje, Henri, Jules, Lukas, Viktor, Louis, Jef, Richard, Hugo, Ernest, Cezar, Kamiel, Nora, Noor, Nelly, Nele, Nikita, Nina, Noë, Noeki, Nutella, Nadia, Nala, Nanoe, Sybil, Basil, Boris, Bikkel, Billie, Fientje, Lilly, Marieke, Oscar, Epke, JP, Radja, Wizzy, Whoopy, Eve, Bruno, Hermes, Hera & Ulysses, bedankt!!